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ON THE MECHANISM OF AMMONIUM ION UPTAKE BY MAIZE ROOTS

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INTRODUCTION

Plants can synthetize a great number of very complex nitrogen compounds such as proteins, nucleic acids, lecithins, chlorophyll, growth substances, etc. from simple substances like ammonium ions and nitrate which are absorbed in the form of inorganic salts from the external environment. Although absorbed nitrogen is for the most part directly assimilated into these organic compounds, some plants show the peculiarity of nitrate accumulation in their tissues, *e.g.* tobacco (*Nicotiana* spec.), sunflower (*Helianthus annuus*) and stinging nettle (*Urtica* spec.).

Ever since JUSTUS VON LIEBIG (1840) suggested that atmospheric ammonia was the main nitrogen source for higher plants, an extensive literature on this subject has appeared. Because soil is a very complex system with many unknown factors, it was difficult to obtain clear information about the mineral requirements of plants while cultivating them in soils. Even the use of sand cultures could not wholly overcome this drawback, since sand may also contain impurities. It was the great merit of SACHS (1860) and KNOP (1860) to introduce the water culture technique, by which it was demonstrated incontestably that nitrogen was absorbed by the roots from their environment in the form of inorganic salts.

Later, the question of whether the nitrate or the ammonium ion is the more preferential nitrogen source was studied in great detail by plant physiologists and agronomists. Most of these numerous investigations, however, have no direct bearing on the subject of this thesis. Only a few publications will be cited in connexion with the so-called "physiologically acid reaction" of ammonium salts, discussed in Chapter II.

Notwithstanding all these studies on nitrogen uptake in particular, and many other investigations concerning the uptake of various mineral elements by plant roots and tissues in general, the mechanism of salt uptake, *i.e.* the mechanism of transfer of salt ions from the medium to the interior of the cell, is still not clearly understood. This thesis attempts to contribute to the knowledge of the mechanism of active salt uptake.

Because many plant roots absorb ammonium ions more rapidly than other ions, the study of ammonium ion uptake offers certain advantages. Moreover, it presents the opportunity to make a quantitative study of the physiologically acid reaction, because this reaction appears to be by far the most conspicious in the absorption of ammonium salts.

Chapter I

HYPOTHESES ON SALT UPTAKE

§ 1. General Statement

Before considering in detail the various hypotheses concerning inorganic salt uptake, it may be useful to state some facts and concepts about salt uptake in general.

(1) Salts are only absorbed in *ionic form.*

This is also true for ammonium ion uptake, although some passive absorption of molecular ammonia may occur under alkaline conditions (*cf.* van den Honert *et al.*, 1955, unpublished).

(2) Salt uptake is *not* a *passive diffusion process* of ions from a higher concentration (external medium) to a lower concentration (root cells). On the contrary, it is an *active process* which transports salts against a concentration gradient. Roots show a particular ability to reduce the external medium to exceedingly low concentrations (*e.g.* phosphate uptake, van den Honert, 1933; and the ammonium ion uptake in the present study). It may be mentioned that Schuffelen (1941, 1942, 1944, 1946) and Vervelde (1952, 1953, 1955) suggested *not* an *active* ion uptake mechanism but a *passive* entrance of ions into the protoplasm. These theories will be discussed in this chapter, *§ 5.*

(3) *Energy* has to be expended to overcome the concentration gradient in salt uptake. This energy is derived from exergonic metabolic processes, *i.e.* in roots by the oxidative breakdown of carbohydrates (root respiration). Therefore, in roots, the presence of oxygen and carbohydrates is a prerequisite for active ion uptake. The necessity of energy-yielding respiratory processes for salt uptake is demonstrated by the fact that, although it is possible to inhibit salt uptake without affecting the respiration, no inhibiter is yet known which stops respiration without stopping salt uptake.

(4) With respect to the *influence of water absorption* on salt uptake, the results obtained by different authors seem contradictory. This discrepancy may be caused partly by different experimental material, *i.e.* peas (Hylmö), broad bean (Brouwer), sugar cane and maize (van den Honert), and partly by different salt concentrations. Probably the influence of the transpiration stream is negligible at low salt concentration (*cf.* van den Honert, 1955*a*), but becomes more marked at the higher salt concentrations (*cf.* Hylmö, 1953 and Brouwer, 1954). In any case, water transport is not essential for an active salt uptake. Submerged aquatic plants, *e.g. Chara, Nitella, Valonia, Halicystis*, as well as storage tissue disks, accumulate ions without appreciable water intake.

It should be emphasized that the various hypotheses on salt uptake discussed below are not strictly comparable. Lundegårdh (1933) suggests a salt uptake mechanism on a molecular level represented by a structural picture indicating the mediating molecules. Steward (1937), however, states more generally a relation between salt uptake and other metabolic processes, without postulating, at that time, a

mechanism of salt uptake. While the carrier hypothesis is an easy concept to handle, it is evidently a simplification of the salt uptake mechanism.

§ 2. The "anion respiration" hypothesis (Lundegårdh)

In 1933 Lundegårdh and Burström developed the "anion respiration" theory, based on experiments with wheat roots. In later years this hypothesis was greatly extended by Lundegårdh and his collaborators. A recent review of this hypothesis is given by Lundegårdh (1954) in a paper at a symposium of the Society of Experimental Biology.

The experimental evidence on which this hypothesis has been based is the following:

(1) Wheat roots showed a negative surface charge in dilute salt solutions. This negative electro-kinetic potential was attributed by Lundegårdh to the dissociation of strong acid groups (pK = 1–2) in the surface membrane of the protoplasm. Anion uptake was considered to be hampered and cation uptake enhanced by this negative charge of the root surface. From this Lundegårdh concluded that, in contrast to cation uptake, anion uptake requires energy.

(2) The transfer of roots from distilled water to a salt solution gave an increase of the respiration rate. The respiration rate in distilled water was called by Lundegårdh "distilled-water respiration" or "ground respiration". Salt uptake was inhibited by cyanide, but the "ground respiration" persisted, being insensitive to cyanide. Therefore, only a cyanide-sensitive component of the aerobic respiration was thought to be related to salt uptake. This suggested the participation of a cytochrome-cytochrome oxidase system in salt uptake.

(3) The uptake of anions showed a linear relationship to respiration, whereas a similar relation did not exist in the case of cations.

From these facts Lundegårdh concluded that there was an anion transport coupled to electron transfer in the cytochrome-cytochrome oxidase system. Thus, in its original form, his hypothesis emphasized an active anion transport only, while cations were moved passively by exchange with acid groups in the protoplasm, along a track parallel to the electrical gradient created by anion transport.

A more detailed statement of this hypothesis and a critical survey of arguments favouring and opposing it are presented in Chapter V.

§ 3. Relationship between metabolism and salt uptake (Steward)

Steward (1937) suggested a relation between salt uptake and such metabolic processes as growth and protein synthesis. The greater the metabolic activity and growth of the tissue involved, the greater was its salt uptake capacity. Later, Hoagland *c.s.* (1939, 1940) accepted Steward's view. As already stressed, the hypotheses of Steward *c.s.* and Lundegårdh are not quite comparable. Steward suggests more generally a correlation between salt uptake and other metabolic processes, whereas Lundegårdh actually formulates a mechanism of salt uptake indicating the mediating groups of molecules. However, in 1947 and

1954 STEWARD extended his hypothesis to include the same molecular level as did Lundegårdh, by assuming that the role of protein synthesis consists of the production of phosphorylated glutamine or γ-glutamyl peptide "carrier" molecules. By the "zwitterion" capacity of these "carrier" molecules, cations as well as anions were bound (or held) and transported through the cytoplasm. Inside, at the vacuole membrane, the glutamine molecules were condensed to protein and might leave the transported ions in a situation in which they could be readily accumulated in the vacuole. Because STEWARD (1954) suggested the mediation of a template surface in this mechanism, he called his hypothesis the "template" hypothesis.

The experimental evidence for a correlation between metabolism and ion uptake was as follows:

(1) Freshly cut potato tuber slices were not immediately capable of accumulating ions, but the capacity to do so developed when the material was suspended for several days in aerated dilute salt solutions. During this treatment there was an increase in the rate of respiration, protein synthesis began, and the cells at the surface of the disk showed a tendency to divide, forming a layer of callus (STEWARD, BERRY, PRESTON and RAMAMURTI; 1943).

A study of the absorption of ions by disks of different thickness led to the conclusion that only the cells at the surface of the slices were involved in metabolic absorption. Further, a close relationship between protein synthesis, respiration rate, and salt uptake was stated. Low respiration was associated with low protein synthesis and low salt uptake, whereas the highest respiration was observed with the highest protein synthesis and salt uptake (STEWARD and PRESTON, 1940, 1941).

(2) Barley roots showed a pronounced longitudinal gradient of salt accumulation. The largest salt uptake occurred at a small distance from the root apex, where the greatest metabolic activity and protein synthesis also took place (PREVOT and STEWARD, 1936).

(3) The salt uptake by barley roots was profoundly influenced by their initial nutritional status and metabolic activity. "Low-salt, high-sugar" roots showed a very great capacity for salt uptake, in contrast to "high-salt, low-sugar" roots, in which the salt uptake capacity was small (HOAGLAND and BROYER, 1936).

As will be shown later (Chapter III), some results of the present study can be connected with the conception of Steward and Hoagland.

§ 4. THE "CARRIER" HYPOTHESIS

This hypothesis is based on the assumption that the entrance of ions into living cells is accompanied by a binding or adsorption to some protoplasmic constituent, *i.e.* to a "carrier" molecule. Fundamentally, this hypothesis is not contradictory to the previously cited hypotheses. LUNDEGÅRDH (1935, 1954) accepted this idea for the cation uptake, whereas Steward's amphoteric glutamine molecule in his "template" hypothesis may be regarded as a carrier. However, for the sake of simplicity, the carrier hypothesis will be discussed separately in this section.

The idea of a binding or adsorption of ions to protoplasmic constituents had already been expressed by OSTERHOUT (1936) and HOAGLAND and BROYER (1936). Such a carrier hypothesis was also suggested by VAN DEN HONERT (1933, 1936), who explained the asymptotical shape of the curve representing the rate of phosphate adsorption in sugar cane by the assumption of a phosphate adsorption or binding to the protoplasm of the root surface. ARISZ (1944, 1945) too, offers arguments in favour of a binding of ions by the protoplasm.

JACOBSON *et al.* (1950) and OVERSTREET *et al.* (1952) extended this hypothesis by the attractive suggestion that ions react with the metabolically produced carrier substances to form complexes which subsequently break down to release free ions according to the equations: $HR + M^+ \rightleftharpoons MR + H^+$ and $ROH + A^- \rightleftharpoons RA + OH^-$.

JACOBSON *et al.* (1950) suggested that in barley roots potassium and hydrogen ions compete for the same site of the carrier, whereas OVERSTREET, JACOBSON and HANDLEY (1952) found evidence for a mutual influence between potassium and calcium uptake. EPSTEIN (1952) and EPSTEIN and HAGEN (1952) have extensively discussed the mode of binding of ions to these postulated carriers. They investigated the effect of varying external concentrations of potassium and sodium on the rate of rubidium uptake by detached barley roots. After a kinetic analysis of their results, they arrived at the conclusion that potassium competes with rubidium for the same site in the postulated carrier, while sodium does not. Applying the same procedure to anions, EPSTEIN (1953) concluded that chloride, but not nitrate, is bound at the same sites as bromide. In addition, HURD-CARRER (1934, 1937, 1938) obtained some evidence in favour of a competition between sulfate and selenate ions for the same binding sites. It may be mentioned here that many other workers in the field of ion uptake agree more or less with the idea of carrier substances (LUNDEGÅRDH, 1954; SCOTT RUSSELL, 1954; SUTCLIFFE, 1954 a, b; CONWAY, 1954).

Little is known about the chemical nature of the postulated carriers. Reference can be made only to the work of COWIE, ROBERTS and ROBERTS (1949) and ROBERTS, ROBERTS and COWIE (1949) on *Escherichia coli*, which showed that hexose phosphate compounds are formed which have a low affinity for sodium and a high affinity for potassium.

In the following sub-sections, some general aspects of this carrier hypothesis such as (1) the *nature of the first binding*, (2) the *place of the first binding*, and (3) the *transport mechanism* will be discussed. All facts known about ion uptake can be placed somewhere in the expanded picture of this hypothesis. It is evident that such a visualization is a simplification of the salt uptake process, but it may well serve as a useful working hypothesis.

(1) The *nature of the first binding*. If the first binding were non-specific, then the driving force in ion uptake would be an electric charge. However, charge seems to play a minor part in ion uptake, since roots accumulate potassium much faster than calcium, and chloride much faster than sulfate. Moreover, VAN DEN HONERT *et al.*

(1953) observed that no other ions could drive phosphate, ammonium ion or nitrate out of their first binding. Therefore, the first binding should be considered as a specific reaction for each ion. This first binding, however, may be partly reversible, as in enzyme reactions.

(2) The *place of the first binding*. The opinions of different authors are contradictory with respect to the location of the place of the first binding in salt uptake. Some investigators suggest a barrier to free diffusion of ions and to exchange processes at the outer surface of the protoplast, while others think it more likely that there is a diffusion barrier in the vinicity of the tonoplast, separating the protoplast from the vacuole in a plant cell.

Van den Honert (1933, 1953, 1955 *b*) suggested a first binding at the very outside of the protoplast "looking through its cell wall windows towards the outside world", because—in contrast to the carbon dioxide intake in photosynthesis—no measurable diffusion resistance between the medium and the place of first binding could be observed. Such a resistance would be evident if it was greater than a water layer of 0.2 mm thickness. (*cf.* van den Honert, 1953).

Holm-Jensen, Krogh and Wartiovaara (1944) studied the potassium and sodium uptake in *Nitella* and *Tolypellopsis* coenocytes with isotopes. They concluded from their experiments that by far the greatest permeability resistance is situated at the outer surface of the protoplast (plasmalemma), whereas the resistance of the vacuole membrane (tonoplast) could only be very small. Moreover Mitchell (1954), using elegant methods, obtained evidence for a phosphate-impermeable barrier at the outer surface of *Staphylococcus* cells.

In contrast to the view stated above, Brooks (1937, 1940) showed that the movement of potassium and sodium ions into the protoplasm of *Nitella* is rapid, whilst subsequent entry in the vacuole takes place slowly and, under anaerobic conditions, perhaps not at all. Also, Arisz (1945) concluded from studies on the ion uptake by *Vallisneria* leaves, that the tonoplast is the region of a cell across which active accumulation occurs. Robertson (1950, 1951) and Sutcliffe (1952) support this conception of a main barrier at the tonoplast, *i.e.* a non-metabolic absorption in the protoplasm and a metabolic absorption in the vacuole.

In the opinion of the present author, some objections can be raised against the latter view. In the first place, one can oppose this view with the following teleological argument: it is unlikely that the protoplast, which so closely regulates its own internal ionic composition, would be freely exposed to the ionic concentrations of its environment. Secondly, the concept of a diffusion barrier and an accumulation mechanism limited solely to the tonoplast is not universally applicable, because animal cells and meristimatic plant cells without a vacuole nevertheless show a highly selective ion-accumulation capacity.

(3) The *transport mechanism*. The first binding at the outside should be followed by an energy-requiring process for the transportation of the bound ions to the interior of the cell. Van den Honert (1933, 1955 *b*) considers the transport mechanism of this carrier system to be comparable to that of a "revolving belt", which is loaded with

ions at the outside surface, carries them inward to deposit them somewhere inside the cell, and then comes back to the surface empty to be reloaded. This simple picture is useful as long as we do not know the true mechanism of carrier transport. According to this revolving-belt picture the rate of ion uptake is determined by three factors:

(a) The *degree of loading* of the belt, *e.g.* expressed in percentage of the total loading capacity. The degree of loading is dependent upon the ion concentration in the medium (rapid equilibrium establishment) and is only very slightly dependent upon temperature. This is true in general for all adsorption equilibria. The loading of the carrier belt may be considered to be a chemical equilibrium reaction similar to that of enzyme reactions.

(b) The *rotation speed* of the belt. The rate of ion intake is assumed to be proportional to the revolving speed of the endless belt. The speed is considered to be greatly dependent on temperature, but it is not affected by the size of its load.

(c) The *loading capacity* of the belt. The maximal loading capacity can be represented by the width of the revolving belt. In the case of nitrate uptake, this capacity is dependent upon the external pH (VAN DEN HONERT, 1955*b*), but for ammonium ion uptake it is not affected by the pH (see the present study).

Ion uptake by intact plants is certainly a catenary process. The processes of carrier mechanism (carrier binding, removal of ions from the protoplasmic boundary inwards), ion transport from cell to cell towards the xylem, ion excretion into the xylem vessels and—following the current view—ion transport to the leaves by means of the transpiration stream (HAAS and REED, 1937; PETRISCHEK, 1953), etc. will occur successively. In such a chain process, the speed of the "slowest" link will finally govern the reaction velocity of the whole process. Now, it is not at all self-evident that the first link, which we have characterized as the carrier mechanism, is the slowest one. However, VAN DEN HONERT (1933, 1937, 1955*b*) has obtained evidence that this is indeed the case for the phosphate uptake by sugar cane and the nitrate uptake by maize. Here, the rate of phosphate or nitrate uptake seems to be determined by factors effective at the outer surface of the protoplasm.

Van den Honert bases his view of a carrier mechanism in direct contact with the medium on the following observations.

(*i*) The shape of the absorption rate-concentration curves of phosphate and nitrate correspond to adsorption isotherms without interference of a diffusion resistance between carrier and medium.

(*ii*) Such a diffusion resistance, if measurable, would become evident at the lowest ion concentrations. However, in phosphate as well as in nitrate absorption, the same temperature coefficients were found in the high and the low concentration range. If at low concentrations a diffusion limited the rate of uptake, much lower temperature coefficients should be expected.

(*iii*) The rate of phosphate uptake in relation to pH can be quantitatively explained by the equilibrium between mono- and di-phosphate

ions as determined by the pH of the medium, on the assumption that only the univalent monophosphate ions are taken up. This points again towards an intimate contact between carrier and medium, because internal and external pH can hardly be supposed to be always identical.

Because external conditions, such as ion concentration and pH, control the rate of ion uptake to such a great extent, the interpretation that the "master reaction" is operating in direct contact with the medium seems to be justified. What we study as the slowest reaction, in measuring the rate of ion uptake by roots of intact plants, may, for this reason, actually be the turnover rate of the carrier mechanism. The circumstance that this mechanism is so easily influenced by environmental factors allows us to study its properties in greater detail.

§ 5. Other hypotheses

The discussion of the results is mainly confined to the three hypotheses mentioned above. It is beyond the scope of this thesis to describe all the other theoretical considerations in this field. However, two hypotheses current in the Netherlands will be briefly mentioned, because both are concerned in the conclusions of the present study.

(1) Schuffelen's hypothesis.

Schuffelen (1941, 1942, 1944, 1946, 1952) attached great importance to the differences in the ionic activity of the environment and of the root content. In his opinion, the ionic activity in the root cells must always be lower than that in the medium in order to permit ion uptake. Furthermore, he gives a mathematical description of the hypothetical equilibrium reactions (Donnan equilibrium) between the outside solution and the root wall. The root wall is thought to be equivalent to the external layer of protoplasm.

Evidently, ion uptake is here taken to be a *passive process*. The sole *active* part of this ion uptake mechanism is the metabolic energy needed to keep the ionic activity in the protoplasm low.

(2) Vervelde's hypothesis.

Vervelde (1952, 1953, 1955) assumed, as a result of root potential measurements, that the principles of a Donnan equilibrium can be applied to the surface layer of the roots because of the presence of non-diffusible anions in the bulk of the protoplasm. On purely theoretical grounds, he proposed a mechanism by which changes in the H^+ion gradient in the interior of the root cells are responsible for salt accumulation. According to his view, three processes are involved in this salt accumulation mechanism. First, the interior of the cell becomes acid due to organic acid production. Subsequently, mineral anions of the medium move inward. Finally, the interior of the cell decreases in acidity, causing cations to move inward and anions to move outward. Salt accumulation is accomplished because the anions tending to move outward are held up by the outer protoplasmic layers with low anion permeability, whereas the cations moving inward are held up by the interior protoplasmic layers where the cation permeability is low.

Here, as in Schuffelen's hypothesis, the actual movement of ions from the medium into the cells is supposed to be a *passive* process. The only energy required for this salt accumulation mechanism is that for the establishment of these H^+ion gradients in the protoplasm. According to this theory, salt uptake depends upon the concentration, mobility, and valency of the ions present in the medium.

The purpose of the experiments described in Chapters II and III was to obtain more information on some special aspects of salt uptake in connexion with the theories cited above. The experiments reported in Chapter II are mainly concerned with the first binding reaction in ion uptake, whereas those described in Chapter III deal with the relationship between respiration and salt uptake.

Chapter II

THE AMMONIUM ION UPTAKE AND HYDROGEN ION RELEASE IN RELATION TO AMMONIUM ION CONCENTRATION AND PH AS OBSERVED IN INTACT MAIZE PLANTS

§ 1. Introductory remarks

For the purpose of studying ammonium ion uptake and hydrogen ion release in relation to the ammonium ion and hydrogen ion concentration of the nutrient solution, experiments were performed with intact maize plants.

A continuous-flow technique for water cultures was used, designed by van den Honert (1933) for his study on the phosphate uptake by sugar cane, at the Sugar Experiment Station at Pasuruan (Java, Indonesia). With this technique it was possible to control accurately the ion composition and pH of the nutrient solution flowing around the roots.

The above technique is based on the principle that a constant flow of nutrient solution will establish a steady state in the root vessel, provided that the solution in this vessel is continuously stirred, because the supply, take-up, and removal of ions per unit of time tend to become constant. An equilibrium is reached when the rate of ion intake by the plant is constant and the water uptake due to transpiration is either nil or constant. It is then possible to measure ion uptake at a constant and adjustable ion concentration and pH. The ammonium ion uptake is computed from the difference which exists between the ammonium ion content of the nutrient solution entering and leaving the root vessel. This ammonium ion uptake can be expressed in mg NH_4^+ ions per hour or alternatively in milli-equivalent (m.e.) NH_4^+ ions per hour. After the establishment of such a steady state, the effluent nutrient solution can be considered as a continuous sample. Therefore, the ion concentrations found in the effluent solution are equal to those existing around the roots.

This and the more detailed description of the apparatus and technique given later (§ 4) show what is meant by ammonium ion uptake and what procedure was applied to measure it. Therefore, only the hydrogen ion release, the so-called "physiologically acid reaction", needs closer attention.

§ 2. The physiologically acid reaction

(1) *Hydrolytically acid reaction versus physiologically acid reaction*

A salt such as ammonium chloride, which is derived from a weak base and a strong acid, produces in aqueous solution an acid reaction called the "hydrolytically acid reaction". If, however, one brings a living plant root into an ammonium chloride solution, then the pH of the solution is lowered to a far greater extent. This phenomenon can be explained by a selective absorption of ammonium ions and subsequent release of hydrogen ions by the root cells. In fact, one can see this as an ion exchange phenomenon. Due to the rapid preferential absorption of ammonium ions compared to chloride ions, the plant root tends to be positively charged against its environment. Consequently, the continuous absorption of ammonium ions tends to be hampered by the repulsion of similar charges. The plant root can only maintain its electro-static equilibrium against the medium by releasing in exchange hydrogen ions or other available cations. In order to distinguish the hydrogen ion increase due to hydrolysis of the salt from that caused by the reaction of the living root to the presence of ammonium salts, the latter is called the "physiologically acid reaction".

(2) *Statement of the problem*

It should then follow that if one were to study the ammonium ion uptake quite apart from the intake of other ions, one should find a 1 : 1 ratio between NH_4^+-ion uptake and H^+-ion release. The aim of this part of the present study is to obtain empirical evidence as to whether or not the ratio of 1 : 1 for $NH_4^+ : H^+$ really exists.

(3) *Other investigations on this subject*

Rautenberg and Kühn (1864) were the first authors to clearly conceive that the physiologically acid reaction of ammonium salts (NH_4Cl) was caused by preferential ammonium ion uptake. These authors drew attention to the possibility that the often-stated superiority of nitrate nitrogen to ammonium nitrogen for plant growth might be due to this physiologically acid reaction, since the acid (HCl) produced in the medium would hamper root growth.

Later, the Russian investigator Prianishnikov (1926, 1951) in particular devoted many qualitative studies to the physiologically acid reaction of ammonium salts. Numerous research workers (*e.g.* Mevius *et al.* (1928, 1930), Pirschle (1930, 1931), Naftel (1931), Loo (1932), Pardo (1932), Clark and Shive (1934), Trelease and Trelease (1935), Arnon *et al.* (1937, 1942 *a*, *b*) and Weissmann (1950, 1951) have investigated the ammonium ion and nitrate uptake in relation to the pH of the medium in various crops. Many of them have made qualitative studies of the physiologically acid action of ammonium salts.

Quantitative investigations of the physiologically acid reaction are confined to the following studies:

Jenny and Cowan (1933) studied the physiologically acid reaction of Ca^{++}ions in a one-ion medium by using adsorbed Ca^{++}ions on clay particles. Such a Ca-clay suspension acts as a one-ion solution, because the clay particles can be considered as non-absorbable anions for the plant. These Ca-clay suspensions were prepared by dialysing or percolating natural clays with strong acids to free them from adsorbed cations. The H^{+}ion-carrying clay obtained in this manner can be converted into a Ca^{++}-clay by the addition of a $Ca(OH)_2$ solution. Soya bean seedlings were cultivated for seven days in Ca-clay suspensions in distilled water. The water loss due to transpiration of the plant was continuous throughout the experiment. The initial pH = 6.3 of the culture solution dropped to pH = 4.3 as a consequence of Ca^{++}-ion uptake. The increase in Ca content of the plants was determined and the amount of hydrogen ion release was estimated by potentiometric titration with $Ca(OH)_2$ to a pH = 6.3.

The data obtained were:

Ca content increase of the plants : 1.020 m.e.
H^{+}-ion release to the clay : 0.948 m.e.

Hence, a stoechiometrical ratio of 93 % between absorbed calcium ions and released hydrogen ions could be observed, *i.e.* nearly two H^{+}ions are released for each Ca^{++}ion absorbed.

Van Raalte (1942, oral communication) performed experiments to determine quantitatively the physiologically acid reaction of ammonium salts. This was done with rice plants in water culture at the Treub laboratory at Bogor (Java, Indonesia). From the observed pH decrease, van Raalte computed the number of hydrogen ions responsible for such a hydrogen ion increase. In this way he showed that about 90 % of the absorbed ammonium ions were exchanged for hydrogen ions.

Conway and O'malley (1946) studied the quantitative relationship between potassium ion uptake and hydrogen ion release in yeast (*Saccharomyces cerevisiae*) cells. Whereas almost no potassium ions are absorbed by resting yeast cells (*cf.* Conway *et al.*, 1950) a rapid potassium ion intake and a nearly equivalent exchange for hydrogen ions occurred during active fermentation. With high potassium concentrations (0.1 – 0.5 N) in the medium, pH values as low as 1.7 – 1.5 could be reached. Actually, these pH values are of the same magnitude as that caused by hydrogen ion secretion of some gastric mucosa (*cf.* Davies and Ogston, 1950). Conway and O'Malley stated that 80 % of the hydrogen ions produced during fermentation were obtained by potassium ion exchange. Therefore, only 20 % of the K^{+}ions seems to enter the cell in association with Cl^{-}ions.

A method of estimating hydrogen ion release quite different from those cited above was used in the present study. It was based on the "base-excess" principle. This idea was suggested by the work of Baas

BECKING (1934, *l.c.* p. 74) on the photosynthesis of marine algae. In order to measure the hydrogen ion release at a constant pH, this base-excess method was employed in connexion with the continuously-flowing water culture technique briefly mentioned in the previous section.

§ 3. PLANT MATERIAL

Maize (*Zea mays* L.) seeds of the single cross hybrid D x 9, obtained from the Plant Selection Station "Centraal Bureau" at Hoofddorp (Holland), were germinated in small clay containers with garden soil. Each seedling was transplanted to a small clay pot when one week old and then to a larger pot (diameter 12 cm), also containing garden soil, at an age of about 3–4 weeks. After 6–8 weeks, these plants had reached a height of 50–75 cm and were suitable for water culture. For this purpose the roots were freed from as much soil as possible by careful washing under running tap water. After that, the plants—usually two together—were placed in culture jars of 400–600 ml capacity containing a WOODFORD and GREGORY (1948) culture solution of the following composition.

NUTRIENT SOLUTION 1

$Ca(NO_3)_2$. 0.102 mM	$MgSO_4$ . . 0.0975 mM
KNO_3 . . . 0.277 mM	KH_2PO_4 . . 0.1505 mM

To this nutrient solution Fe was added in the form of ferric tartrate to a concentration of 1 p.p.m. To avoid micronutrient deficiency, 1 ml HOAGLAND and SNYDER (1933) A–Z micronutrient solution was supplied per 1 litre Woodford and Gregory nutrient solution. The culture solutions were continuously aerated with compressed air and renewed twice a week.

The original roots, to some of which soil still adhered, were gradually cleared away, until only the newly-developed roots remained. In contrast to the original roots, these new roots were well adapted to the water culture medium. In the normal growing season the new roots developed very vigorously and in about 3–4 weeks the original root system was completely replaced. This procedure for raising mature maize plants suitable for water culture, however, can not be followed the whole year round, since the growing season of maize is confined to the months April to September. Experience showed that these maize plants could only be used for the experiments three months after germination. Therefore, it was only profitable to germinate new plants between early April and early June. Plants germinated early in June could be transferred to water culture at the end of August or early September. Plants germinated later than June could not be used because of their insufficient root development. Nevertheless, plants which already possessed newly developed roots remained usable for ion absorption experiments up to the end of October.

§ 4. APPARATUS AND TECHNIQUE

A detailed description of the apparatus may be useful, because the original description by VAN DEN HONERT (1933 a) was published in a

less accessible journal. Moreover, some modifications in technique are introduced in the present study. The arrangement for constant-flow in water cultures is shown in Figure 1.

Three stock-solution reservoirs (A,B,C,) of 60 litre capacity were placed on a table at a height of 2 m. These large containers were first painted black and then white, in order to avoid algal growth and heating by the sun. Because the fluid level inside the reservoir could not be checked, a U-shaped glass tube (1) was connected to the reservoirs and the liquid level read from this. The nutrient solutions employed were previously prepared in a similar 60 litre container (D) standing on ground level. This vessel was calibrated and provided with a Bunsen valve (2) by means of which the nutrient solution could be pumped upwards to the reservoirs A, B and C with compressed air or a bicycle pump.

The nutrient solutions in these reservoirs contained a basic salt solution (see Table) to which an ammonium salt in the ammonium solution reservoir B, an acid in the acid solution reservoir A, and a bicarbonate in the alkaline solution reservoir C, was added. Thus, the nutrient solutions employed had the following composition.

NUTRIENT SOLUTION 2

Composition	concentration in p.p.m.		
	Reservoir A Acid solution	Reservoir B Ammonium solution	Reservoir C Alkaline solution
K_2SO_4	20.00	20.00	20.00
$MgSO_4.7\ H_2O$ . .	5.00	5.00	5.00
$CaCl_2.0\ H_2O$. . .	5.00	5.00	5.00
H_3BO_3	0.04	0.04	0.04
KH_2PO_4	5.00	5.00	0
NH_4Cl	0	237.78	0
HCl	0.002 N	0	0
$NaHCO_3$	0	0	0.004 N

The tabulation of nutrient solution 2 shows that all ion concentrations—except those of the ammonium ions—are extremely low. It may be emphasized that this is one of the advantages of the constant-flow technique in water culture. Because of the constant supply of new ions, the plant could withdraw ions from a low, but constantly replenished, concentration level. The NH_4^+ion concentration of the ammonium stock solution was 80 p.p.m. Since the investigation concerned the physiologically acid reaction, the normality of the alkaline solution was made twice that of the acid solution. KH_2PO_4 was not added to the alkaline bicarbonate solution, because it would have lowered the pH. In order to avoid iron chlorosis in the plants, Fe was added in the form of a synthetic Fe-humate (*cf.* VAN DEN HONERT, 1933*a*, *l.c.*

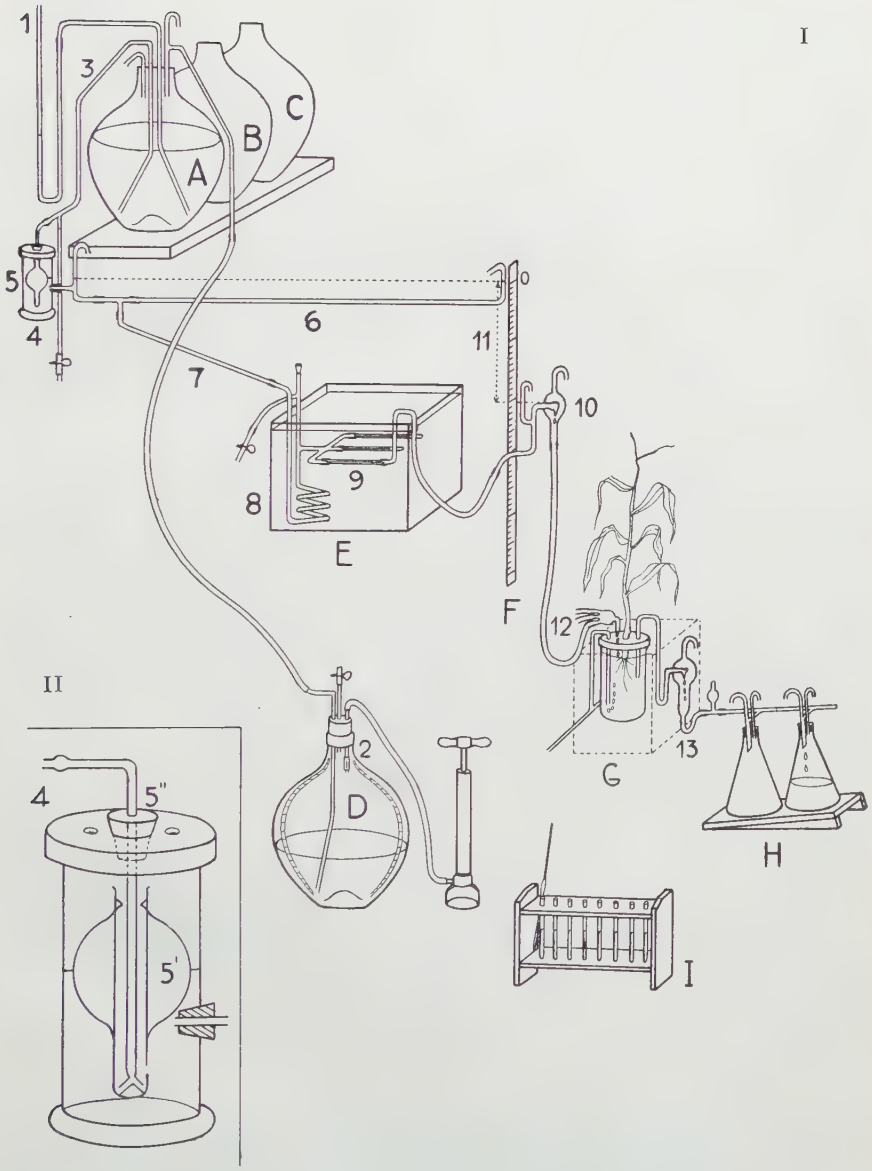


Fig. 1. Apparatus for constant-flow in water culture.

I. General view of the constant-flow arrangement. Arrangement drawn schematically and not to scale.

II. In detail: Constant-level glass cylinder with float device, drawn to scale (diameter of the bulb = 5.5 cm).

p. 85). One ml of this Fe-humate solution was added to the nutrient solution in the root vessels twice a week.

The nutrient solution of each stock-solution reservoir was connected by a glass siphon (3) to a glass cylinder (4). This cylinder contained a glass float designed in such a way that it kept the nutrient solution in the glass cylinder at a constant level. A detailed picture of such a float is represented in Figure 1 (II). A float consists of an air-filled glass bulb (5′) functioning as floating body and provided at its lower end with a conical ground-glass joint which closes off the outlet tube of the siphon (3) when the nutrient solution in the cylinder has reached a certain level. A glass tube (6) connected the cylinder to a wooden rack placed in front of the reservoirs. The constant fluid level in the cylinder could then be read on a graduated scale attached to the rack. By changing the outlet level of the siphon (3), which could be accomplished by sliding its extension tube along a cork (5″), this constant fluid level could be made to coincide with the zero-line of the scale.

The nutrient solution leaving the constant-level cylinder flowed through a tube (7) to a glass spiral (8) and then to three glass capillary tubes (9). From each one it then flowed through a plastic tube to a small overflow funnel (10). From this funnel it dripped slowly through plastic tubing into the root vessel (G) placed in a water thermostat.

The rate of flow of the nutrient solution into the root vessel is dependent, on the one hand, on the difference in level (see 11) between the zero-line and the position of the overflow funnel (10) and, on the other hand, on the resistance met by the fluid between constant-level cylinder (4) and overflow funnel (10). The rate of flow (i) of the nutrient solution can be represented by the formula:

$$i = \frac{p_1 - p_2}{r},$$

wherein (p_1–p_2) = the pressure difference between 0-level and overflow funnel level, and r = the resistance operating between glass cylinder and overflow funnel. Now, according to the law of POISEUILLE the resistance (r) met by a liquid slowly moving through a tube is proportional to the length of the tube (l) and inversely proportional to the fourth power of the tube's radius (a), *i.e.*:

$$r = \frac{8\, l\, \mu}{\pi\, a^4},$$

in which μ = the viscosity coefficient of the liquid.

The glass and plastic tubing had a comparatively much larger bore (7–10 mm) than the capillary tubes (0.5 mm). Therefore, by far the greatest resistance was situated in the capillaries, and the rate of flow of the whole system was governed by the resistance of these capillary tubes. This can be demonstrated by an example derived from the conditions prevailing in the apparatus. The resistance met by a liquid in 3 meters of tubing with a bore of 10 mm is only 0.000075 of that in a capillary tube of 25 cm length and a bore of 0.5 mm. Thus, the

resistance encountered by the liquid in other parts of the apparatus was negligible compared to the resistance of the capillary tubes.

The viscosity coefficient of water decreases 2 to 3 % with a temperature increase of 1°C. Therefore, to obtain a constant rate of flow, it was necessary to keep the nutrient solution passing through the capillary tubes at a constant temperature. This was attained by placing the capillaries in a water thermostat (E) of 20°C ± 0.1°C and by passing the solution through a spiral before it entered the capillary tubes.

As it was almost impossible to construct capillary tubes of exactly the same resistance, the rate of flow obtained with each capillary had to be verified empirically. This was done by fastening a strip of millimetre paper to the wooden rack (F). Subsequently, the rate of flow of each capillary at various levels (11) of the overflow funnel, *e.g.* 30, 60, and 90 cm, was determined by collecting the effluent solution in a volumetric flask and measuring the time necessary to fill it. The relation between the rate of flow and the level difference corresponding to it could be plotted graphically. This relationship was linear, and by linear interpolation a whole graduated scale of flow-rates could be drawn. These values were marked on a tinned-iron strip in ml per hour units with an intercept of 1 ml per hour.

The solutions delivered by the overflow funnels (10) from the three stock solution reservoirs were mixed in a glass distributer (12), before the final mixture dripped slowly into the root vessel. The ammonium ion content of the nutrient solution entering the root vessel was a function of the dilution by the other stock solutions. The NH_4^+–ion content of the solution in reservoir B was 80 p.p.m. All NH_4^+ concentrations below this could be obtained by dilution with appropriate volumes of acid and alkaline solution. Also, at a constant NH_4^+ concentration, the pH of the nutrient solution could be changed by altering the ratio of acid to alkaline solution supplied, provided that their total volume remained constant.

An air stream introduced into the root vessel vigorously mixed the entering solution with the nutrient solution already present. The same air stream also furnished the roots with the indispensable oxygen supply. To prevent water losses by evaporation, the air stream was previously saturated with water vapour by passing it through a washing bottle containing distilled water. In order to measure the ion uptake at a constant root temperature, the root vessel (G) was placed in a water thermostat of 20°C ± 0.1°C.

The nutrient solution discharged from the root vessel through an overflow funnel (13) was collected in Erlenmeyer flasks (H) of about 1 litre capacity which were connected in series. These flasks were filled consecutively due to their position on a gradually sloping shelf. The nutrient solution collected in these flasks could be sampled and analysed for its ion content. The total output of nutrient solution could be measured, since the flasks were calibrated and the solution volume in the last flask could be determined by weighing. The time required for filling the flasks was also measured, thus the rate of flow of the nutrient

solution leaving the root vessel could be computed. From the difference in flow-rate of the solution entering and leaving the root vessel, the average rate of water absorption could be found. In the calculation of the ion uptake, the measured NH_4^+–ion content of the effluent solution was corrected for the concentration increase due to water uptake by the root system.

The conditions in the apparatus must be chosen in such a way that a new equilibrium concentration can be reached in a short time. This was promoted by a high rate of flow of the nutrient solution. On the other hand, the concentration difference between the nutrient solution entering and leaving the root vessel must be large enough to enable a sufficiently accurate estimation of the rate of NH_4^+–ion uptake. The latter condition was facilitated by a slow flow-rate of the nutrient solution. Since satisfying both requisites produced opposite effects, a compromise had to be sought. For this, it was desirable to distinguish the factors concerned in establishing a new equilibrium condition. Some of these factors played a part in the apparatus, and they were chosen so as to obtain a new steady state in as short time as possible.

A theoretical calculation of the time interval necessary between two measurements was found to be very helpful. The factors governing the rate at which a new equilibrium was established were partly mechanical, and partly influenced by the rate of ion uptake by the plant. In the following calculation the experimental time required to reach a new equilibrium was computed for a flow-system where there is a flow of nutrient solution through the apparatus with no plant in the root vessel.

Let A = capacity of the root vessel, c_0 = initial concentration in the root vessel, c = concentration in the root vessel at time t, E = concentration of the entering liquid and V = rate of flow of the entering liquid. It is necessary to determine the time required to go from c_0 to E.

$$dc = \frac{E\,V\,dt}{A} - \frac{c\,V\,dt}{A} \tag{1}$$

Because the concentration itself (c) is of no interest, but the concentration difference ($E - c$) is, this variable is substituted in equation (1):

$$\frac{dc}{dt} = \frac{-d(E-c)}{dt} = \frac{V}{A}(E-c). \tag{2}$$

This equation gives, after integration:

$$\ln(E-c) = -\frac{V}{A}t + C. \tag{3}$$

In order to solve C, use is made of the special case, $c = c_0$ and $t = 0$:

$$\ln(E - c_0) = C.$$

Substitution in equation (3) gives:

$$\ln(E-c) = -\frac{V}{A}t + \ln(E-c_0)$$

$$\frac{V}{A}t = \ln(E-c_0) - \ln(E-c) = \ln\frac{E-c_0}{E-c}$$

$$t = \frac{A}{V}\ln\frac{E-c_0}{E-c}. \tag{4}$$

If $c = E$, then $t = \frac{A}{V}\ln\frac{E-c_0}{0} = \frac{A}{V}\infty$

It is evident from equation (4), that the time required to approach a new equilibrium condition is inversely proportional to the rate of flow (V), and directly proportional to the volume of the root vessel (A) and to the natural logarithm of the ratio between initial concentration difference and final concentration difference. As was to be expected, c becomes equal to E only after an infinite time.

With the root systems employed and a root vessel of a 2-litre capacity, it was found empirically that a rate of flow of nutrient solution of 200 ml/hr was the most favourable. The conditions used furnish the following example: A = 2000 ml, V = 200 ml/hr, c_0 = 1 p.p.m. NH_4^+, and E = 20 p.p.m. NH_4^+ ("standard condition", see p. 21). The time required to attain a 98 % correct final concentration, *i.e.* c = 0.98 E, is:

$$t = \frac{2000 \text{ ml}}{200 \text{ ml/hr}} \ln \frac{19 \text{ p.p.m.}}{(20\text{–}19.6) \text{ p.p.m.}} = 38.6 \text{ hours.}$$

Thus, 3.86 x the volume of the root vessel has to be circulated before the final concentration differs not more than 2 % from the ion concentration of the entering solution. *Cf.* VAN DEN HONERT (1930, pp. 209-210) whose value, from an analogous calculation concerning CO_2 assimilation, was 3.91 x the volume of the assimilation vessel.

Assuming that the ion uptake-concentration relation corresponds to a Langmuir equation (Chapter IV), where p = proportional to the total amount carrier and m = Michaelis–Menten constant, then:

$$\frac{dc}{dt} = \frac{VE}{A} - \frac{Vc}{A} - \frac{p}{A} \frac{c}{c+m} \tag{5}$$

If a plant is immersed in the root vessel, in contrast to a mechanical flow-system, the concentration of the entering liquid (E) will never be equal to the final concentration (c_∞). Therefore, c_∞ is substituted for E. When an equilibrium is established, then $\frac{dc}{dt} = 0$ and the concentration in the root vessel will be c_∞.

$$0 = \frac{V}{A}(E - c_\infty) - \frac{p}{A} \frac{c_\infty}{c_\infty + m}$$

Subtracting this equation from equation (5) gives:

$$\frac{dc}{dt} = \frac{V}{A}(c_\infty - c) + \frac{p}{A}\left(\frac{c_\infty}{c_\infty + m} - \frac{c}{c+m}\right) \tag{6}$$

which equation may be compared to equation (2) where $E = c_\infty$. As the first term in both equations is the same and the second term has the same sign, it is evident that the presence of a plant will always shorten the time required to reach a new equilibrium. This is not so obvious at first sight: for example, in going from high initial concentration in the root vessel to low final concentration, a plant will speed up the establishment of a new equilibrium concentration by its ion absorption capacity. However, in the case of going from low initial to high final concentration, a plant seems to be doing just the reverse, as it counteracts by its ion uptake the increase in concentration. Yet, in either case, a plant will shorten the time required to establish a new equilibrium, as can be seen from the above calculation.

This can be understood because analysis shows that the total process consists of two opposing factors. One of these is the concentration change induced by the plant's ion uptake. The other is the concentration range involved in reaching the final concentration c_∞. In going from low initial to high final concentration, the plant slows down the concentration increase by its ion uptake, but the concentration range is shortened with (E-c_∞). In this case, the second factor dominates the first, while in the reverse case (*i.e.* going from high initial to low final concentration) the first dominates the second.

From the previous considerations, it is evident that a time of less than 38.6 hours was necessary to reach a new steady state. Therefore, a time period of 24–36 hours between experiments was used. The establishment of a new equilibrium was usually checked empirically: the NH_4^+ concentration will be the same in each successive flask

collecting the discharged nutrient solution if equilibrium has been reached.

The pH value of the nutrient solution in the root vessel was estimated colorimetrically by comparison with buffer solutions of known pH (McIlvaine citric acid-phosphate buffers; Clark, 1928). For every pH-range a standard comparison scale of buffers in 6 mm bore test tubes was mounted in a test tube rack (Fig. 1, I). In the present study the following pH standard series were used: pH = 3.4–4.2 (indicator bromo-phenol blue, colour change pH = 3.8); pH = 4.0–5.0 (bromo-cresol green, pH = 4.5); pH = 5.2–6.8 (bromo-cresol purple, pH = 6.0); and pH = 6.8–8.0 (phenol red, pH = 7.3). One ml buffer solution and 1 or 2 droplets of indicator were employed. Toluene was added to the buffer solutions in order to avoid fungus growth.

It must be emphasized that the carbon dioxide production by the roots had a profound influence on the pH of the medium around the roots. Since the medium was only slightly buffered, care was taken not to bring the sample into contact with air, because the loss of even a little CO_2 would cause the pH to shift appreciably (0.2–0.4 pH units) towards the alkaline side. For this reason, the following procedure for measuring pH was employed. Indicator solution was first put into the test tubes. The sample was pipetted into the test tube keeping the tip of the pipette below the surface of the solution. In this way carbon dioxide could not escape and the indicator could be homogeneously mixed with the solution.

§ 5. Modifications of the technique

In subsequent NH_4^+–ion absorption experiments (§ 8, IV) at low pH values, it was desirable to alter to some extent the flow technique as well as the composition of the nutrient solution used.

It was indicated in the preceding section that the alkaline stock solution did not contain the acidly dissociated KH_2PO_4. Consequently changes in the ratio of the amount of acid and alkaline solution necessary for the adjustment of the pH would also change the potassium and phosphate concentration. This disadvantage could be eliminated by the use of four stock-solution reservoirs instead of three. With this device, the acid and alkaline stock solutions consisted of only acid and bicarbonate, respectively, in distilled water. Further, it was necessary to increase the Ca content of the nutrient solution, since ammonium nutrition at low pH values seems to be injurious to the root, especially with low Ca content in the nutrient solution. This agrees with the observations of Jacobson and Swanback (1933) that ammonium nitrogen hampers the uptake of Ca and Mg ions in tobacco. Hewitt (1947) found that in kale, sugar beet, and tomato plants grown in sand, Ca deficiency developed in the presence of ammonium nitrogen.

The chloride ion in the nutrient solution was replaced by the sulfate ion, since sulfate is less readily absorbed by maize roots than chloride. This is an advantage, because of less interference to the physiologically acid reaction by NH_4^+–ion uptake. To make the analyses even more

PLATE I

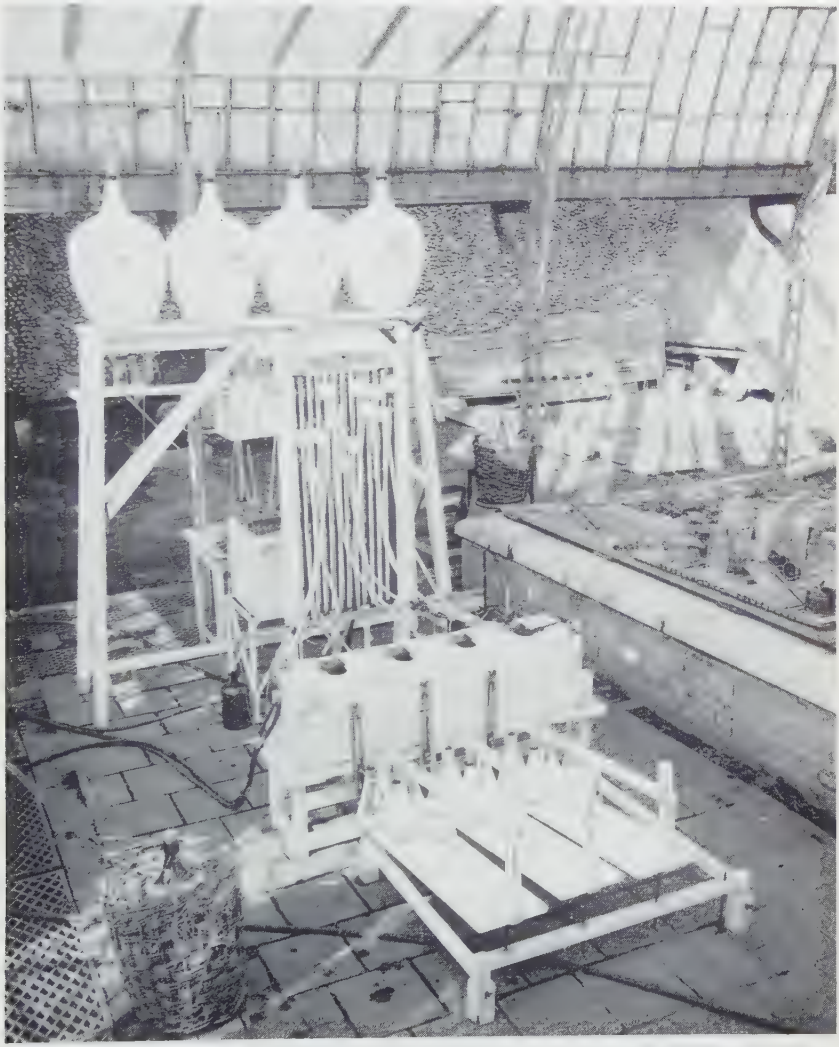


PLATE I

Constant-flow water culture apparatus, without accessory equipment. Viewed from lower right to upper left: in the foreground, the Erlenmeyer flasks standing on a tilted platform. Behind them, the water thermostat containing the root vessels. Above the thermostat, the rack with the overflow funnels. Behind this rack, the water thermostat for the capillary tubes. Above and to the rear, shelf with the constant-level cylinders and, just above them, the four stock solution reservoirs (*cf.* Fig. 2, p. 15 and pp. 13–20).

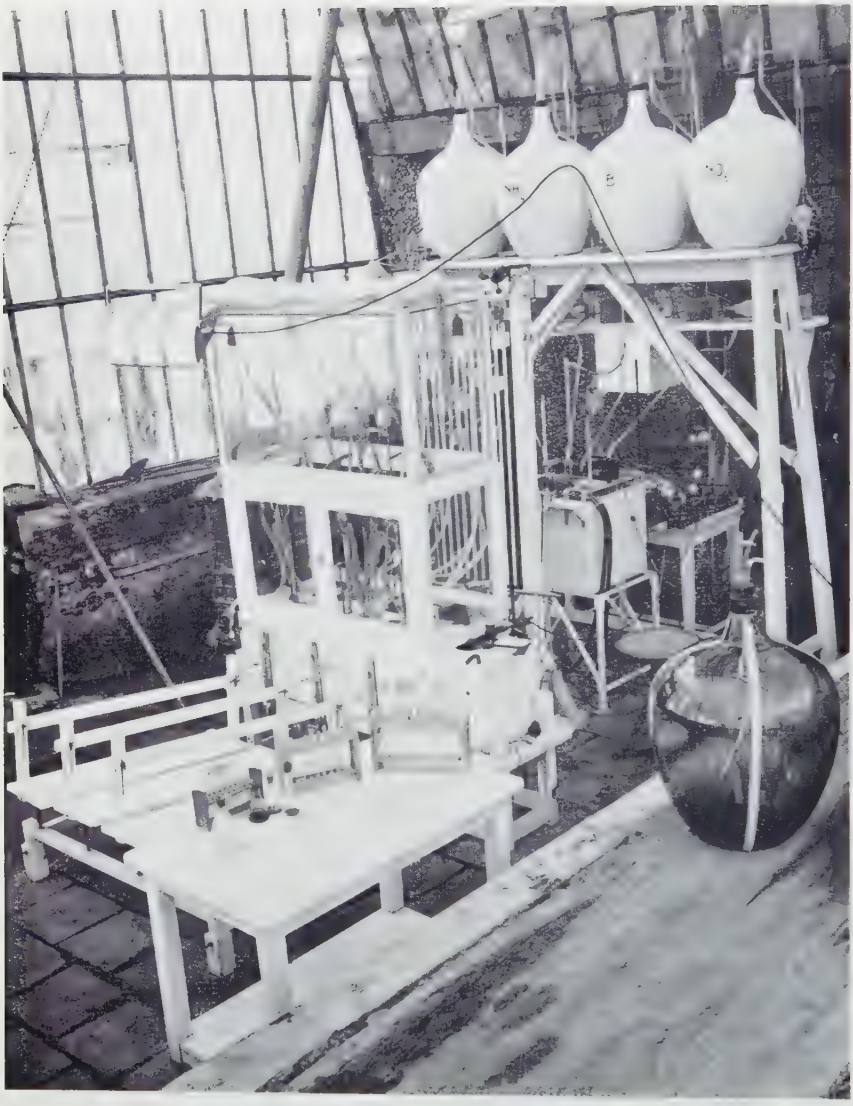


PLATE II

Constant-flow water culture apparatus, with accessory equipment. Cabinet containing plants permits control of air temperature and humidity. High-pressure mercury-vapour lamp (Philips HO 450 W: 20,000 lumen) provides regulation of day length. On the table on the foreground, test tube racks containing the buffer solutions used as comparison standards. At right, calibrated 60-L container for preparation of nutrient solutions (*cf.* Fig. 2, p. 15 and pp. 13–20).

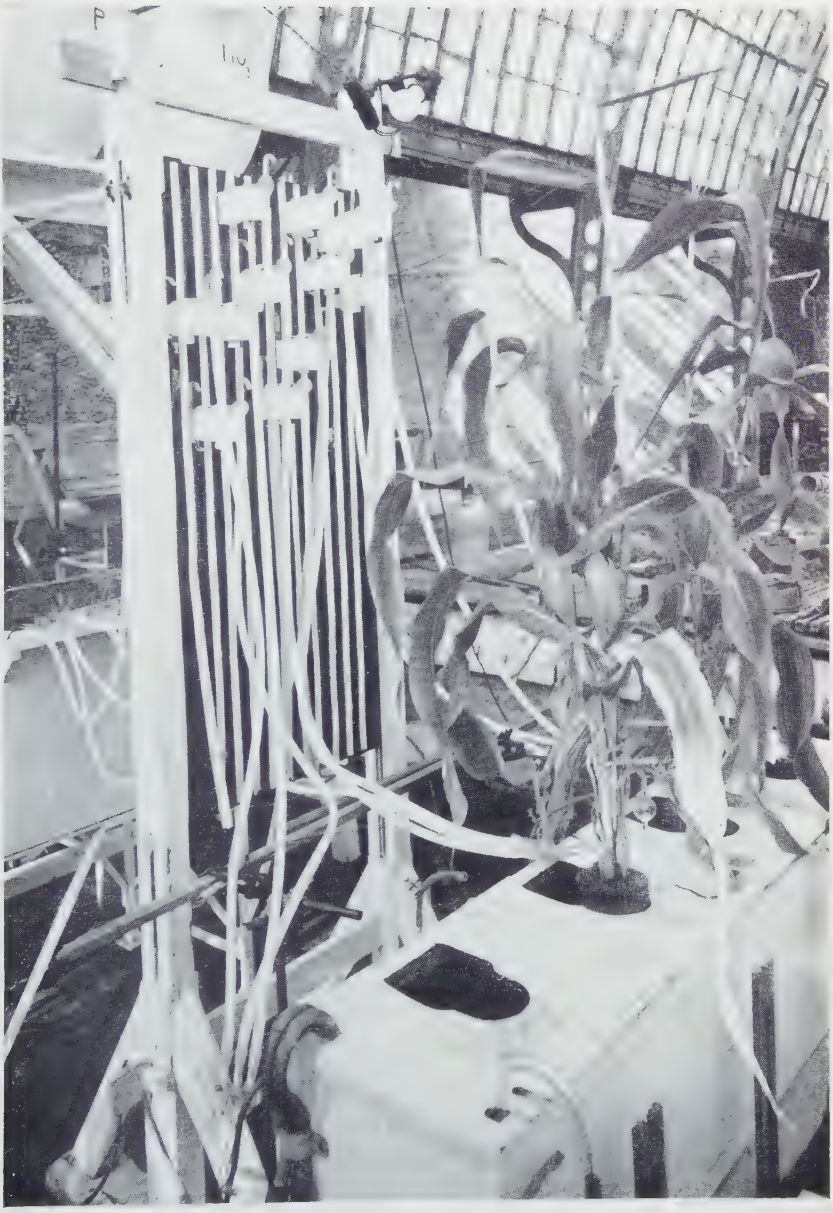


PLATE III

Full-grown maize plants, with ripening ears, in position in the root vessels of the constant flow water culture apparatus. From the reservoirs, the nutrient solutions delivered by the overflow funnels are brought together by plastic tubing and mixed in a glass distributor tube before the final mixture drips into the root vessels (*cf.* p. 17).

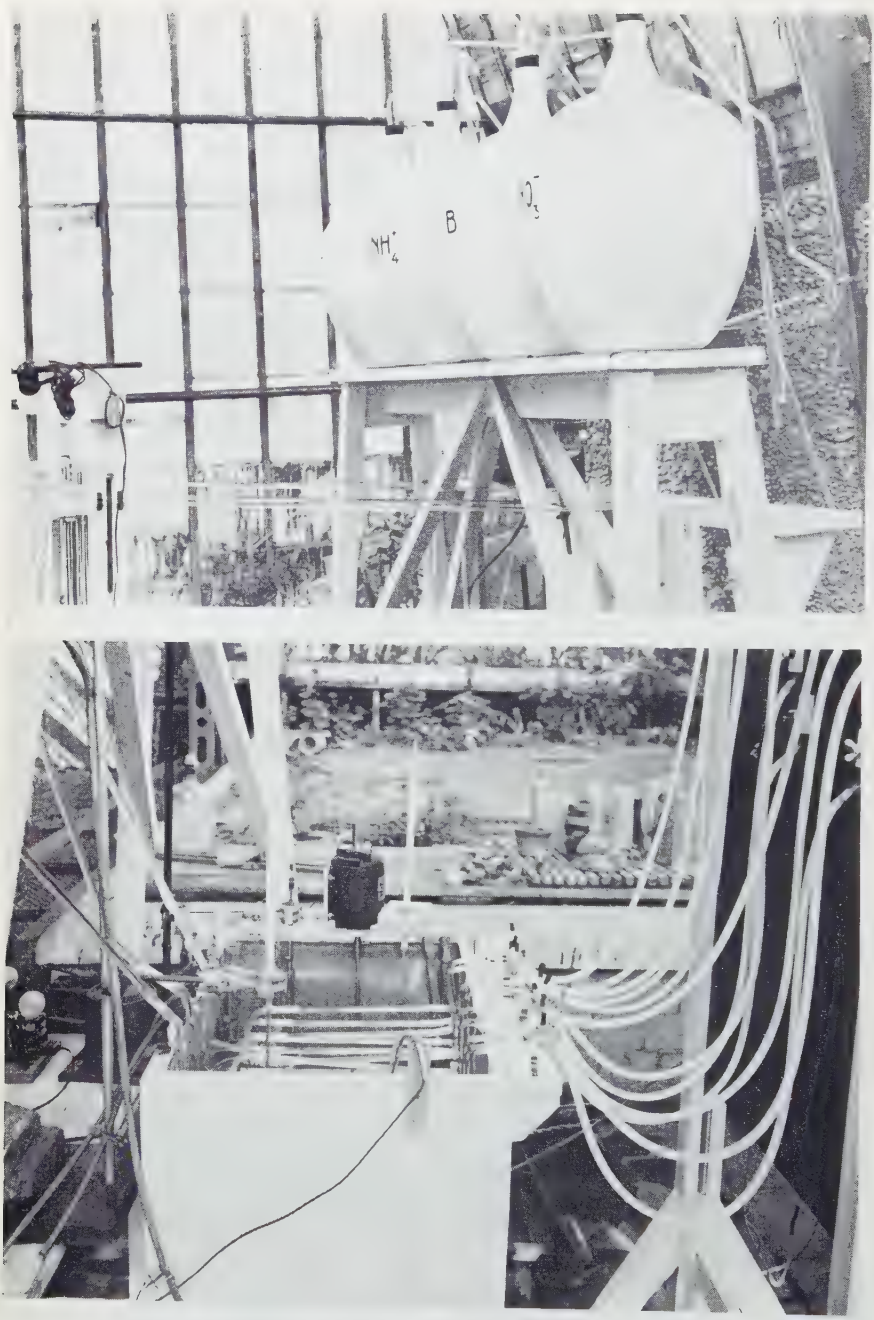
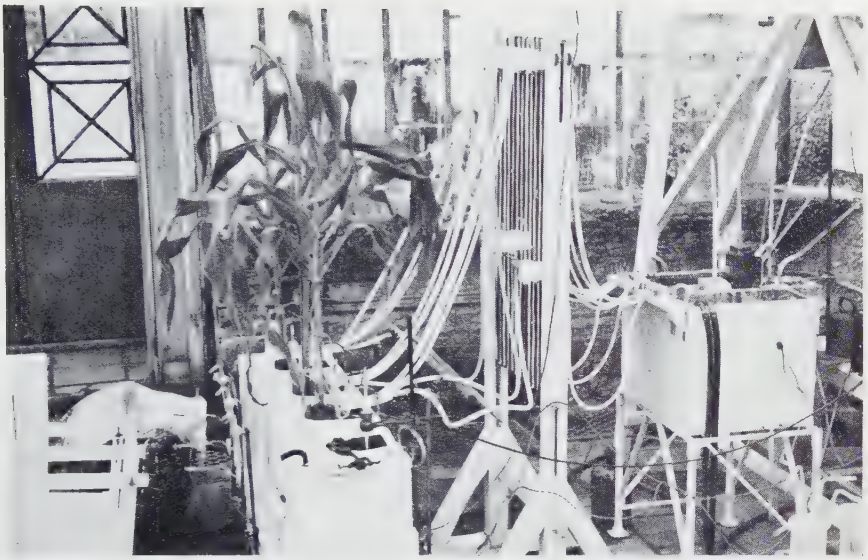


PLATE IV

Above: The four 60-L stock solution reservoirs in place on the 2 m – high platform. The horizontal glass tubes lead the liquid from the four constant-level glass cylinders to the overflow funnel rack, thus providing the zero-line of the calibrated rate-of-flow scales (*cf.* pp. 14 and 16).

Below: View of water thermostat and glass capillary tubes which provide a constant rate of flow of the nutrient solution. Also visible in the thermostat: heating spiral (in front) and water-cooling pipes (in back), the toluene thermoregulator, the electric stirring mechanism, and two thermometers (*cf.* pp. 16–17).



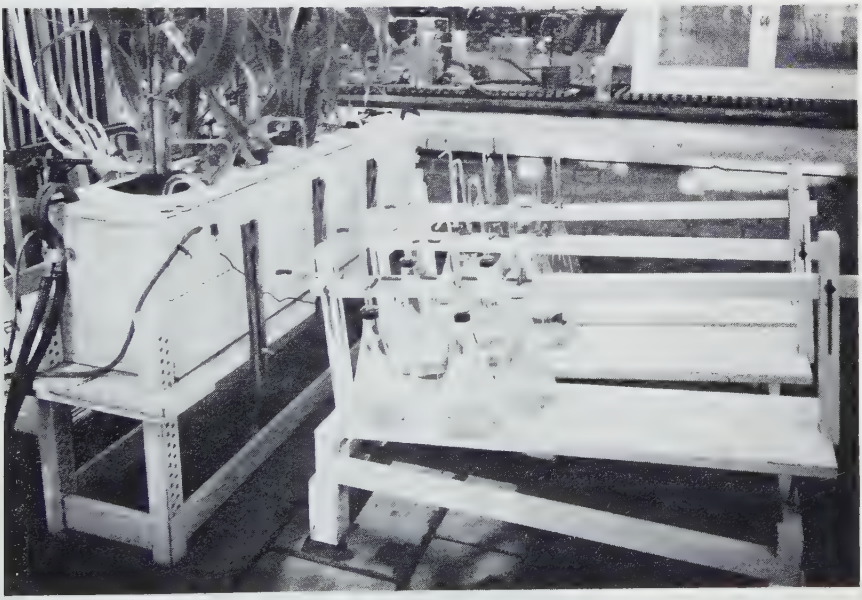


PLATE V

Above: Side view of constant-flow water culture apparatus. Condition of the vigorously developing young plants, here 1.25 m high, demonstrates the adaptability of maize to this type of culture (*cf.* pp. 13–17).

Below: Detailed view of several series of 1-L flasks collecting the effluent nutrient solution discharged from the root vessels by overflow funnels. The slope on which the flasks stand causes them to fill successively, thus providing separate samples for given time intervals. At extreme left, the water-cooling pipes mounted on the thermostat containing the root vessels; to the right of these pipes, the electric heating spiral is partially visible (*cf.* p. 17).

simple, the potassium was replaced by sodium. The final composition of the nutrient solution was as follows:

NUTRIENT SOLUTION 3

Composition	concentration in p.p.m.		normality	
	Reservoir A Salt solution	Reservoir B Ammonium solution	Reservoir C Acid solution	Reservoir D Alkaline solution
Na_2SO_4	40.00	40.00	0	0
$MgSO_4 . 7\ H_2O$ . .	10.00	10.00	0	0
$CaSO_4 . 2\ H_2O$ . .	20.00	20.00	0	0
H_3BO_3	0.08	0.08	0	0
NaH_2PO_4	10.00	10.00	0	0
$(NH_4)_2SO_4$ . . .	0	586.96	0	0
H_2SO_4	0	0	0.004 N	0
$NaHCO_3$	0	0	0	0.008 N

A comparison of this nutrient solution with that previously used (nutrient solution 2) shows that all salt concentrations are doubled. This doubling was the consequence of the new technique, in which by a two-fold dilution, the final solution was obtained. For example, to effect a total rate of flow of 200 ml/hr the amount of acid plus alkaline solution and that of ammonium plus salt solution were both kept at 100 ml/hr. The combination of these solutions thus resulted in a total rate of flow of 200 ml/hr. As the acid and the alkaline solutions did not contain the basic salt solution, the final mixture had only half the initial concentration of each salt. As a result, changes in the ratio of acid and alkaline solution did not affect the potassium and phosphate concentration. Moreover, the modified technique had the advantage that the acid and alkaline stock solutions remained stable much longer, since they did not contain nutrient salts.

§ 6. The relative rate of salt uptake

The actively absorbing area of roots varies considerably in different plants due to unequal root development. Moreover, this area in any given plant will change because of root growth and root decay. For this reason, it is necessary to have a relative unit of absorption in order to compare data. Since the actively-absorbing area of a root is not proportional to its weight, root weights cannot be used as relative unit.

Therefore, as suggested by van den Honert (1933) in his study on the phosphate uptake of sugar cane, the ammonium ion uptake itself, under an arbitrarily chosen "standard condition", was made the relative unit. This "standard condition" was defined as the rate of NH_4^+–ion uptake at a NH_4^+ concentration of 10-20 p.p.m., a pH = 6.0, and a root temperature of 20°C. The NH_4^+ concentration could

be so widely indicated because within this concentration range the NH_4^+–ion uptake had already approximately reached its maximal value.

The NH_4^+–ion uptake measurements made under conditions other (*i.e.* with respect to NH_4^+ concentration and/or pH) than the "standard condition" were always fixed between two ion uptake measurements made under the "standard condition". By linear interpolation in time, the standard uptake value at the point of time of the non-standard measurement was found. The points on the graphs represent the ratio between non-standard uptake value and standard uptake value. Because the standard uptake value is the relative unit of uptake, all standard values have the value 1.0 in the graphs.

§ 7. Analytical methods

The following analytical methods for the determination of ammonium, potassium, and *sodium* ion uptake, as well as hydrogen ion release, were applied.

1. Ammonia determination

For the quantitative determination of the ammonium ion content, the Nessler colorimetric method was used (Allport, 1947, p. 382).

The determination was performed in the following way. To 25 ml of nutrient solution containing ammonium ions, 0.5 ml of 50 % sodium-potassium tartrate was added to avoid precipitates of Ca and Mg after the addition of the strongly alkaline Nessler reagent. Then, 1 ml of Nessler reagent was added and the solution thoroughly mixed with the aid of a glass rod. After 15 minutes, when the maximal colour intensity had developed, the optical density of the solution was measured by means of a Unicam (Model SP 350) spectrophotometer. A standard curve of the relation between optical density and NH_4^+ concentration was obtained with standard solutions containing 0.1 to 1.0 p.p.m. NH_4^+. In this concentration range the extinction was linear with respect to the concentration (Lambert–Beer law). The measurements were conducted at a wave length of 440 mμ. The NH_4^+–ion content of the test solution was determined with the aid of the standard curve. The NH_4^+–ion concentration of the nutrient solution was brought by dilution within the limits of the standard curve. Depending on the rate of dilution, the accuracy obtained was of the order of 2–4 %.

2. Potassium and sodium determinations

The potassium and sodium determinations were made with a Beckman flame photometer. With this technique, a 1-ml aliquot of the nutrient solution containing K or Na is sprayed by means of an air stream into an oxygen-butane flame. The intensity of light emitted by the elements was measured with a DU quartz spectrophotometer. In the present study a permanent standard curve relating light intensity of the emission spectrum and ion concentration was not obtainable as the gas pressure, and in consequence of this the flame temperature,

was insufficiently constant. Therefore, prepared standard solutions with known K^+ and Na^+ concentration were measured simultaneously with the solution of unknown concentration. The ion content of the unknown solution was always fixed between that of two of the standard solutions. Although a graph of the ion concentration plotted against light emission intensity gave a hyperbolic curve, a linear interpolation was applied. This was sufficiently accurate because the concentration difference between both standard solutions used was small, *i.e.* 1 p.p.m. for potassium and 2–3 p.p.m. for sodium.

All K and Na determinations were made in a complete nutrient solution. A marked mutual interference between the light emission of the various elements, *e.g.* between potassium and sodium, was observed. Therefore, one-salt standard solutions did not give reliable results. The standard solutions had to contain not only the varying ion in question but also the interfering ion, in about the same concentration as in the nutrient solution. The K and Na determinations were made at a wave length of 767 mμ and 589 mμ respectively. The analyses had an accuracy of about 3–5 %.

3. Determination of the hydrogen release

The hydrogen ion release was determined by means of the difference in base-excess of the nutrient solution entering and leaving the root vessel. The base-excess concept and estimation procedure was discussed by Johnston (1916) and Buch (1932). The "base-excess" concept can be best explained by a simplified example.

A. The concept of base-excess

(a) If to a volume of distilled water 10 ml of 0.1 N HCl is added and the liquid boiled for a moment and then cooled, exactly 10 ml 0.1 N KOH is necessary to reneutralize the solution. The same is true if the solution is titrated back to a pH = 3.6, provided a correction is introduced for the systematic titration error caused by the back-titration to this low end-point. This situation does not alter if, instead of distilled water, a solution of neutral salts (*e.g.* KCl, $NaNO_3$, $CaCl_2$ or $MgSO_4$) is used. Nor will the presence of an ammonium salt change this situation, since the hydrolytically acid reaction caused by an ammonium salt is still negligible at pH = 3.6.

(b) However, the situation will change considerably if the solution contains carbonic acid salts. As carbonates are in this special case of no interest, though they are important in sea water, the discussion will be confined to the bicarbonate-carbonic acid buffer system. So, if the solution contains $Na^+HCO_3^-$, the addition of 10 ml of 0.1 N HCl and the subsequent boiling will remove CO_2. After the addition of 10 ml 0.1 N KOH, there will be an excess of OH^- ions equivalent to the amount of HCO_3^- previously present in the solution. In this way the name "base-excess" (*cf.* Johnston, 1916) for those metal ions which are present in the form of carbonic acid salts, can be easily understood.

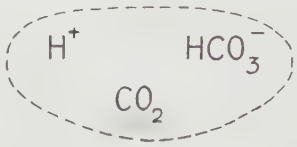
(c) Hence, by adding HCl and titrating back with KOH, the

amount of OH^-ions equivalent to the original amount HCO_3^-ions, and thus the quantity of base-excess, can be determined. Back-titration to an end-point pH = 3.6 is desirable: a solution containing an ammonium salt should not allowed to become alkaline, since NH_3 would escape and this would decrease the amount of base-excess. It is also preferable to keep the solution outside the buffer range of the phosphates present.

B. *The influence of the plant on the amount of base-excess*

(a) The amount of base-excess is not altered, if
(i) the plant absorbs equivalent amounts of cations and anions from the nutrient solution, *e.g.* equivalents amounts of K^+ and Cl^-ions.
(ii) the plant releases equivalent amounts of cations and anions from the root tissue, *e.g.* equivalent amounts of K^+ and Cl^-ions.

(b) The addition of carbon dioxide produced by the root respiration will certainly alter the pH of the nutrient solution, but it will not change the amount of base-excess. The amount of base-excess is zero in a K^+Cl^- solution. Now add carbon dioxide to this solution:



After the addition of mineral acid and subsequent boiling, the chemical species represented within the dashed line will be driven out, but the amount of base-excess will remain zero.

(c) The amount of base-excess will *decrease*, if NH_4^+ or K^+ions are exchanged for H^+ions. A decrease of the amount of base-excess will occur because the H^+ions produced will bind HCO_3^-ions and thus carbon dioxide will escape. The amount of base-excess will *increase*, if Cl^- or NO_3^-ions are exchanged for OH^- or HCO_3^-ions.

(d) The method for determining base-excess cannot be used for estimating the hydrogen ion release if HCO_3^-ions are also absorbed by the roots. However, experiments of Lundegårdh (1933; 1937, p. 108) and Hoagland (1944, p. 132) made it seem likely that roots actively producing carbon dioxide do not absorb HCO_3^-ions.

(e) If the root system of the plant absorbs water due to *transpiration* by the upper parts, the solution will become more concentrated. The sample volume of nutrient solution withdrawn for the base-excess determination should be corrected for water uptake by the plant.

(f) After applying this volume correction, one should find, therefore, exactly the same amount of base-excess, independent of the carbon dioxide production by the roots, as long as the roots absorb or release cations and anions in equivalent amounts. However, the amount of base-excess will change if cations and anions are absorbed or released in unequal amount.

C. *Determination procedure*

The hydrogen ion release by the roots was computed from the base-excess difference of the nutrient solution entering and leaving the root vessel.

The solution *discharged* from the root vessel was collected in conical flasks and could be sampled from these flasks. The *entering* solution was sampled, either by collecting it directly before it dripped into the root vessel, or indirectly by drawing off (see I, 4, Fig. 1) the nutrient solution from the reservoirs and recomposing it by uniting volume parts of the three solutions in the same ratio as indicated by their rates of flow. The solutions obtained by both methods did not differ essentially as was shown by an integral checking of the apparatus, *i.e.* the apparatus without plants in the root vessels.

The base-excess determination procedure was as follows: To a 50-ml aliquot nutrient solution, 10 ml 0.02 N HCl was added and the solution then boiled, while a steady current of carbon dioxide-free air (obtained by passing over soda-lime) was bubbled through the solution. After cooling, the solution was titrated against 0.02 N NaOH. A comparison scale was made of citric acid-phosphate buffer mixtures with a pH range of 3.2–4.2 (with phenol blue indicator). This was done in order to titrate the solution entering and leaving the root vessel to exactly the same end-point of pH = 3.6. All determinations were made in duplicate and the average value was taken. A standard error of $\pm$ 1.11 x 10^{-3}m.e./hr was calculated for the hydrogen ion release data presented in the tables.

§ 8. Experiments

I. *Preliminary experiments on the relation between ammonium ion uptake and hydrogen ion release*

As previously explained (§ 6), the NH_4^+–ion uptake data had to be expressed in relative units in order to make all data mutually comparable. Because of this, the H^+–ion release had also to be given in relative units. In Figures 2 and 3, the ratio between H^+–ion release and NH_4^+–ion uptake is plotted against the NH_4^+ concentration, *i.e.* each relative NH_4^+–ion uptake value is shown with its corresponding relative H^+–ion release value.

In order to give the reader an impression of the absolute magnitude of the NH_4^+–ion uptake and H^+–ion release, some of these data are represented in Table 1.

From Table 1 it appears that under the given experimental conditions practically all NH_4^+ ions are absorbed from the entering nutrient solution when its NH_4^+–ion content is 10 p.p.m. As has been stated before, the nutrient solution leaving the root vessel (column 2) is identical with the nutrient solution surrounding the roots. The 200 ml/hr rate of flow was favourable for obtaining a large concentration difference between the solutions entering and leaving the root vessel. It is clear from column 6 that these nearly full-grown maize plants absorb the considerable quantity of about 2.0 mg NH_4^+/hour per plant.

In these experiments, the H^+–ion release (the physiologically acid reaction) was very appreciable, being 150 x 10^{-3}m.e./hour per plant. Compared to the calculated standard error of $\pm$ 1.11 x 10^{-3}m.e./hour, the H^+–ion release figures were very high. The ratio H^+/NH_4^+ (last column) had an average value of 1.32 when the NH_4^+ concentration was between 0.1–0.2 p.p.m. In other words, 32 % more H^+ions were released than NH_4^+ions were taken up. This situation was possibly due to a H^+-ion release due to K^+, Na^+ and Ca^{++}ion uptake, which was not taken into account in these experiments. In order to eliminate this interfering factor, in subsequent experiments K^+ and Na^+ determinations were performed and the H^+–ion release figures were corrected for the uptake of these ions.

TABLE 1

NH_4^+–ion *uptake and* H^+*–ion release in three nearly full-grown maize plants* A, B, C. Experiments made for three consecutive days (9/8–11/8.1952) using nutrient solution 2 with a rate of flow of 200 ml/hr, at pH = 6.0 and root temperature 20° C.

Plant	NH_4^+ mg/L	NH_4^+ mg/L out	water absorption ml/hr	NH_4^+ mg/hr in	NH_4^+ mg/hr out	NH_4^+ absorption mg/hr	NH_4^+ absorption m.e. 10^{-3}hr	H^+ release m.e. 10^{-3}hr	ratio $\frac{H^+}{NH_4^+}$
A	10.02	0.100	4.9	2.004	0.020	1.984	109.98	127.54	1.16
B	10.02	0.102	8.1	2.004	0.019	1.985	110.03	137.87	1.25
C	9.55	0.146	13.2	2.004	0.029	1.975	109.48	145.70	1.33
A	10.02	0.068	10.3	2.004	0.013	1.991	110.37	149.34	1.35
B	10.02	0.200	12.2	2.004	0.038	1.966	108.98	155.01	1.42
C	9.55	0.218	16.0	2.004	0.042	1.962	108.76	151.45	1.39
A	10.02	0.094	13.6	2.004	0.018	1.986	110.09	154.19	1.40
B	10.02	0.119	15.7	2.004	0.022	1.982	109.87	141.29	1.29
C	9.55	0.170	17.7	2.004	0.033	1.971	109.26	150.00	1.38

The third column of Table 1 shows the rate of water absorption by the root systems. This absorption rate ranged between 5–18 ml/hour, *i.e.* about 2.5–9.0 % of the rate of flow of the nutrient solution. Because such a water absorption had considerably increased the ion concentration of the nutrient solution, a correction for water absorption was introduced. From the figures in Table 1, it is apparent that the water uptake had no appreciable effect on the rate of NH_4^+–ion uptake. This is in agreement with the observations of VAN DEN HONERT *et al.* (1955 *a*). The water absorption rates varied in the different plants according to different transpiration rates influenced by unequal shoot development. Moreover, the rate of water absorption varied according to temperature and humidity changes in the greenhouse: high temperature and low relative humidity increased transpiration, which considerably accelerated the water absorption rate of the root systems.

II. *The physiologically acid reaction of the nutrient solution without ammonium ions*

The physiologically acid reaction due to ammonium ion uptake can only be determined if the uptake of other ions is made as small as possible. It must be emphasized that the base-excess method only measures the net effect of cation and anion uptake. Therefore, the most accurate results could be expected from the use of one-ion solutions (NH_4^+-ions absorbed on clay particles or on synthetic ion exchange resins) or one-salt solutions (pure ammonium salt solutions). However, such unbalanced nutrient solutions proved to be injurious to roots, particularly because the experiments were conducted over a period of several weeks. It is well known that a calcium-free medium is especially toxic for roots.

Because of the long experimental time required in the present experiments, a nutrient solution containing all essential ions for growth was indispensable. Notwithstanding this fact, it was possible to select conditions under which the H^+–ion release could be attributed mainly to NH_4^+–ion uptake. First, the ion concentrations of all accompanying ions indispensable for growth, such as potassium, phosphate, calcium and magnesium, were kept as low as possible. This condition was especially favoured by the continuous-flow technique. No deficit in these ions could occur; due to the constant supply of new ions, the plant could absorb from a low, but continuously replenished, ion level. In the nutrient solutions used (nutrient solutions 2 and 3), the NH_4^+ concentration was always much higher than that of the accompanying cations. Secondly, for the non-essential salts, ions were chosen which were known to be absorbed at a relatively slow rate. Accordingly, $NaHCO_3$ was used for the alkaline solution, and HCl or H_2SO_4 for the acid solution, since Na^+, HCO_3^-, Cl^- or $SO_4^=$ ions are taken up slowly or not at all (HCO_3^-) by maize roots.

TABLE 2

The physiologically acid reaction of a complete nutrient solution where the NH_4^+ *ions were replaced by* Na^+ *ions.* The H^+–ion release and the K^+-ion uptake were determined on two consecutive days (2/9–3/9.1952) for three plants (D, E, F) using nutrient solution 2 with 200 ml/hr rate of flow, at pH = 6.0 and root temperature 20° C.

Plant	K^+		K^+		K^+		H^+ release m.e. 10^{-3}hr	ratio $\frac{H^+}{K^+}$
	mg/L in	mg/L out	mg/hr in	mg/hr out	absorption mg/hr	absorption m.e. 10^{-3}hr		
D	9.86	7.84	1.97	1.57	0.40	10.23	14.22	1.39
E	9.87	8.84	1.98	1.77	0.21	5.37	9.84	1.77
F	9.87	8.85	1.98	1.77	0.21	5.37	6.48	1.21
D	9.90	8.07	1.98	1.62	0.36	9.21	9.48	1.03
E	9.90	8.66	1.98	1.73	0.25	6.39	7.11	1.11
F	9.90	8.80	1.98	1.76	0.22	5.63	4.74	0.84

The unavoidable uptake of ions other than NH_4^+ interfered with the NH_4^+uptake – H^+release relationship. The amount of such residual interference was determined. Omission of only the ammonium salt was not advisable since that would concomitantly decrease the concentration of the anions. Therefore, in the complete nutrient solution the ammonium ions normally present were replaced by sodium ions, *i.e.* the $(NH_4)_2SO_4$ in the ammonium-ion stock solution was replaced by an equimolar quantity of Na_2SO_4. In this way, the physiologically acid reaction of NH_4^+ ions could be compared with that of Na^+ ions. The sole ion left in the nutrient solution which was taken up by maize at an appreciable rate was potassium. Therefore, it was desirable to determine the K^+–ion uptake and to correct the measured H^+–ion release for the H^+–ion exchange due to K^+–ion uptake.

A comparison of Table 1 with Table 2 shows that the physiologically acid reaction of a nutrient solution, where the NH_4^+ ions were replaced by Na^+ ions, is only 4–5 % (maximum 10 %) of the physiologically acid reaction of a nutrient solution containing 0.1–0.2 p.p.m. NH_4^+. Moreover, it appears from the figures that the observed physiologically acid reaction is of about the same magnitude as that of the K^+–ion uptake. In the last column of Table 2, it is seen that normally more H^+ions are released than K^+ions are taken up. This discrepancy can be explained by the uptake of calcium and sodium ions, for which the H^+–ion release values were not corrected. However, by correcting the hydrogen ion release for potassium uptake, a physiologically acid reaction, almost entirely due to ammonium ion uptake, is obtained. Hence, in all subsequent experiments the potassium ion uptake was determined in addition to the ammonium ion uptake, and the base-excess value was corrected for potassium ion uptake.

III. *The relation between ammonium ion concentration, and the relative rate of ammonium ion uptake and hydrogen ion release at pH = 6.0 and root temperature 20°C*

In this series of experiments, in contrast to those described in Section I, the ammonium-ion uptake was expressed in relative units (*cf.* § 6).

Vigorously growing, nearly-mature maize plants were used (*cf.* § 3). Nutrient solution 2 was used with a rate of flow of 200 ml/hour. The nutrient solution was kept at pH = 6.0 and root temperature 20°C. The K^+–ion uptake was determined, and the H^+–ion release values were corrected for the H^+–ion exchange caused by K^+–ion absorption.

From Fig. 2, it is evident that after reaching a NH_4^+ concentration of 10 p.p.m. the relative rate of NH_4^+–ion uptake increases only negligibly with an increase of the NH_4^+ concentration in the medium. For this reason, the relative uptake-concentration curve has the shape of a hyperbola. The "half value" concentration, that is, the concentration where 50 % of the maximum rate of NH_4^+–ion uptake is reached (a value comparable to the Michaelis–Menten enzyme constant), lies at a NH_4^+ concentration of 0.23 p.p.m.

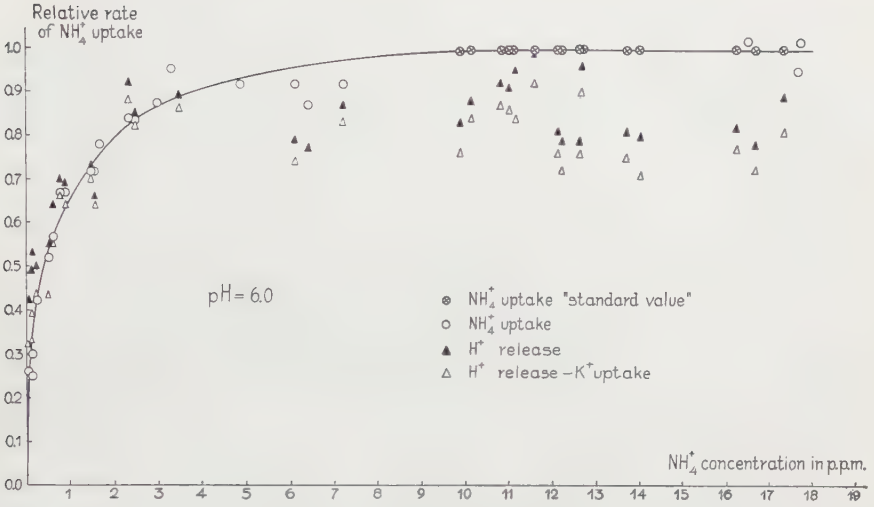


Fig. 2. Relation between ammonium ion concentration, and the relative rate of ammonium ion uptake (circles) and hydrogen ion release (triangles) at pH = 6.0 and 20° C. Data obtained from 9–23 September 1952.

Moreover, Fig. 2 shows that the H^+–ion release below a NH_4^+ concentration of 3 p.p.m. is about 100 % of the NH_4^+–ion uptake, *i.e.* for each NH_4^+ion taken up one H^+ion is released. At very low NH_4^+concentrations, slightly more H^+ions are released. This can be explained by a certain amount of Ca^{++}– and Na^+–ion uptake for which the hydrogen ion release figures were not corrected. In contrast to this observation, the H^+–ion release was only 75–80 % of the NH_4^+–ion uptake at the higher NH_4^+ concentrations (above 3 p.p.m.). The H^+–ion release tends to decrease at still higher NH_4^+ concentrations, *e.g.* 20 p.p.m. An increased NH_4^+ concentration also implies an increased anion concentration, because this increase is obtained by ammonium salt addition. Therefore, the greater interference with the hydrogen ion release at higher ammonium salt concentrations can be explained by an increased anion absorption (giving OH^-–ion release) while the NH_4^+–ion uptake has already approximately reached its maximum value.

IV. *The relation between ammonium ion concentration and the relative rate of ammonium ion uptake and hydrogen ion release at pH = 4.6 and root temperature 20°C*

In these series, the relative rate of NH_4^+–ion absorption and H^+–ion release was studied at a pH = 4.6 and a root temperature of 20°C.

Here too, nearly-mature maize plants, first reared in garden soil and subsequently transferred to water cultures, were used (*cf.* § 3). The rate of flow of the nutrient solution was 200 ml/hour. For the experiments, a K^+–free nutrient solution (nutrient solution 3) was

employed and consequently only Na^+–determinations were necessary. The H^+–ion release values obtained were corrected for the H^+–ion release due to Na^+–ion uptake. All relative absorption rate values mentioned were obtained by taking the ratio between the absolute absorption value at pH = 4.6 and the absorption value under standard conditions (*cf.* § 6).

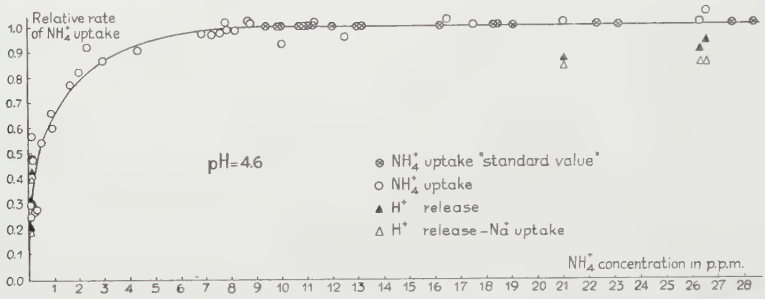


Fig. 3. Relation between ammonium ion concentration, and the relative rate of ammonium ion absorption (circles) and hydrogen ion release (triangles) at pH = 4.6 and 20° C. Data obtained between 15 August to 15 October 1954.

A comparison of Figures 2 and 3 shows that there is no significant difference between the relative rate of ammonium ion absorption at pH = 6.0 and at pH = 4.6, nor did the data for the six experiments on the hydrogen ion release show appreciable differences at the same pH values. However, experiments performed at pH = 4.0 gave quite another picture. About twenty experiments (not presented here) conducted at this low pH, showed a considerably lower NH_4^+–ion uptake than those at pH = 6.0. This diminished NH_4^+–ion uptake was caused by damage to the maize roots, which seem to be very sensitive to low pH values. Therefore, these data have no significance relative to the NH_4^+–ion uptake problem as discussed here. However, it does merit attention that in spite of the toxic effect of these low pH values on the root tissue, no appreciable difference in the ratio between H^+–ion release and NH_4^+–ion absorption was observed.

V. *The effect of prolonged ammonium nutrition on the rate of ammonium ion uptake*

In these experiments, maize plants taken from a Woodford and Gregory nutrient solution (nutrient solution 1) containing only nitrate nitrogen were transferred to solutions (nutrient solutions 2 and 3) containing ammonium nitrogen. As a rule, it could be observed that the NH_4^+–ion absorption by the roots increased during the first 24 hours. This phenomenon could be explained by an adaption of these nitrate-reared roots to ammonium nutrition.

However, in the subsequent days and weeks (the time depending on whether a low or high NH_4^+ concentration was applied), there was a gradual decrease in the NH_4^+–ion uptake capacity of the roots

which, with the constant-flow arrangement for water culture used in these experiments, could be followed readily from day to day. One example will be cited in Table 3 as an illustration. This series indicates the maximum rate of NH_4^+–ion absorption by the root systems employed, because care was taken to measure the uptake in the asymptotic part of the uptake-concentration curve (*i.e.* in a range where the concentration has only little influence on the rate of ion absorption).

TABLE 3

NH^+–ion *absorption in three different plants* (H, I, J) *measured at two-day intervals for 4 days.* The NH_4^+–ion uptake can be assumed to be maximal because relatively high NH_4^+ concentrations were applied. Nutrient solution 2 was used with a rate of flow of 200 ml/hr, at pH = 6.0 and root temperature 20° C.

Date	NH_4^+–ion uptake in m.e. 10^{-3}/hr		
	Plant H	Plant I	Plant J
18/9–1952	155.77	168.51	110.31
20/9–1952	125.83	143.02	94.24
22/9–1952	97.56	124.17	83.15

The decrease in NH_4^+–ion absorption rate after prolonged ammonium nutrition may have some connexion with concomitantly observed morphological deformations of the roots. Secondary maize roots previously grown in a nitrate-containing nutrient solution (Woodford and Gregory nutrient solution) show only limited growth after transfer to an ammonium-containing nutrient solution. The growth of the apical meristems of these roots is soon checked and numerous new side-roots develop over the whole length of the root. However, the development of these new apical meristems is checked in its turn and so the process repeats itself.

Fig. 4. Deformation of secondary maize roots (thick roots of the second order) by ammonium nutrition. The inhibited development of the root-tip meristems and numerous newly-formed, swollen, side branches can be noted. Figure drawn from a photograph.

Apart from the inhibition of the activity of the apical meristems, these ammonium-poisoned maize roots also show root tips which are more or less swollen. This gives the roots the appearance of being lined with short spiny side roots, and in advanced cases the roots may show coralline or tuber-like deformations. In contrast to this picture, maize roots developed in nutrient solutions containing nitrate nitrogen are slender and only a little ramificated. A characteristic picture of ammonium-poisoning symptoms in maize roots grown first in a nitrate nitrogen solution and then transferred to an ammonium nitrogen solution is given in Fig. 4.

It may be remarked that van den Honert (personal communication, and KONINGSBERGER and VAN DEN HONERT, 1931) obtained quite similar ammonium-poisoning symptoms in sugar cane roots in water culture. Here too, the roots showed an excessive branching and stunting.

VI. *The influence of the season and the age of the plant on the rate of ammonium ion uptake and hydrogen ion release*

In section V it is stated that the NH_4^+–ion uptake capacity of maize roots decreases gradually as a result of ammonium-poisoning, a decrease which is especially pronounced when the roots absorb NH_4^+ ions at a maximal rate for a prolonged period. It may be emphasized that this decrease in NH_4^+–ion absorption rate is more rapid when the maize plants involved are older and grow less vigorously.

It is worth mentioning that in these roots of old maize plants the NH_4^+–ion uptake diminishes while the Na^+–ion uptake remains at about the same level as in vigorously growing plants. In the latter, the NH_4^+–ion uptake at a NH_4^+concentration of 10 p.p.m. is about 15 times as great as the Na^+–ion uptake at a concentration of 35 p.p.m. Na^+. In somewhat older plants, this ratio decreases to 10 : 1, whereas with further aging it gradually decreases to as low as 3 : 1. This observation indicates a difference in the mechanism of NH_4^+–ion and Na^+–ion uptake.

In older maize plants, the NH_4^+–ion absorption rate is about 40–50 % of the initial absorption rate. Moreover, in these roots the ratio between H^+–ion release and NH^+–ion absorption changes considerably. In vigorously growing maize plants, the rate of H^+–ion release at the higher NH_4^+ concentrations is about 80 % of the NH_4^+–ion absorption rate. However, this H^+–ion release ratio decreases gradually to only 30 % in aged maize roots. In a special experimental series this phenomenon was studied in greater detail with the same batch of maize plants (germinated in soil in May and transferred to a Woodford and Gregory nutrient solution early in August) at different ages of the plants. During a period of two months, using fresh plants each time, the ratio of H^+–ion release to NH_4^+–ion uptake was determined with the constant-flow water culture technique. The experiments were performed at a relatively high NH_4^+ concentration to have approximately the maximal rate of NH_4^+–ion uptake.

TABLE 4

Ratio between H^+*–ion release and* NH_4^+*–ion uptake in the same set of maize plants at varying ages.* Plants germinated in soil in May and transferred to a Woodford and Gregory nutrient solution in early August. For the experiments nutrient solution 2 was used with a rate of flow of 200 ml/hr, pH = 6.0 and root temperature 20° C.

1952	ratio H^+/NH_4^+	No. of exps.
September	0.86 ± 0.02	15
Early October . .	0.75 ± 0.03	10
End October . . .	0.32 ± 0.04	15

In agreement with the preliminary data, this series shows a decrease in H^+–ion release of about 50 %. This decrease in H^+–ion release in old maize plants can be caused either by an increase in anion uptake or by a cation release. An investigation of this problem indicated that in these older maize roots the anion uptake was of approximately the same magnitude as that in roots of vigorously growing plants. However, in the old plants there was a considerable K^+–ion release, *i.e.* 1–2 p.p.m. K^+/hour per plant. Therefore, it is clear that in old maize roots there is a considerable exchange of NH_4^+ions for K^+ions during NH_4^+–ion uptake. Potassium release by roots has been frequently reported in the literature (*cf.* Sekera, 1928; Luttkus and Böttcher, 1939; Humphries, 1950, 1951, 1952).

Chapter III

THE EFFECT OF SALTS, ESPECIALLY AMMONIUM SALTS, ON THE ROOT RESPIRATION OF EXCISED MAIZE ROOTS

§ 1. Introductory remarks

Whenever salt is accumulated or actively transported by living cells, their metabolism will inevitably be involved. As already mentioned in Chapter II, fully developed, older maize plants show a much smaller capacity to accumulate ammonium ions than active growing plants. Moreover, the ammonium ion absorption capacity of old plants declines steadily. The impression is formed that these old maize plants are soon "saturated" with ammonium ions due to the non-utilization of these ions for synthesis in growth. In contrast to this, actively growing maize plants show a more constant ammonium ion uptake, because ammonium ion utilization in protein synthesis seems to keep up with the absorption, resulting in a "steady state". A quite similar phenomenon can be observed in maize seedlings in water culture, where not only the ammonium ion uptake but also the nitrate, potassium, and sodium uptake decline rapidly after a large initial uptake. Here too, this "saturation" or "indigestion" effect can be accounted for by a lack of sufficient utilization of these ions in metabolism.

The same evidence has been recorded by several investigators. Van den Honert (1933) reported an "indigestion" effect in sugar cane after a previous high phosphate absorption. Hoagland *et al.* (1936, 1944) stated more generally that the much lower ion uptake obtained after an initial high salt intake is due to the "high-salt" condition of the tissue involved. Also, Sutcliffe (1952, 1954) found that the potassium accumulation capacity of beet root disks is profoundly influenced by their potassium content.

Apparently there is a relation between salt accumulation and the metabolic status of the plant, *i.e.* its capacity to synthesize, and its salt and carbohydrate content. The question arises whether there exists a similar relation between salt respiration and the metabolic status of the tissue. With respect to these problems, the effect of salts and carbohydrates on the respiration of roots pretreated in different ways was investigated.

The experiments were performed with excised roots of pretreated maize plants. Special attention was paid to cases of deficiencies in special mineral elements and to the specific response of the respiration after supplying these ions. In special connexion with Lundegårdh's hypothesis, the influence of cation absorption on respiration in the absence of absorbable anions was likewise investigated. The experiments were divided into the following subjects:

I. The effect of salts, especially ammonium salts, on the root respiration of excised maize roots.

II. The effect of sugars with or without simultaneous addition of ammonium salts or nitrates on the root respiration, and the determination of the respiratory quotient, in normal and in nitrogen-starved maize roots.

III. The effect of NH_4^+-bearing ion exchange resins on the root respiration of nitrogen-starved maize plants.

IV. The effect of phosphate on the root respiration of phosphate-starved maize plants.

§ 2. Material and methods

Maize seed of the single-cross hybrid D x 9, obtained from the Plant Selection Station "Centraal Bureau" at Hoofddorp (Holland), was germinated between two layers of humid filter paper on a pad of wet cotton in large open Petri dishes (diameter 25 cm). After a germination time of approximately a week, the seedlings were removed from the dishes and fastened by means of melted paraffine to waxed wire nettings. These nettings with the seedlings were placed in 600-ml culture vessels containing nutrient solution. In contrast to the previous experiments with full-grown maize plants, the seedlings were not placed in a Woodford and Gregory (1948) nutrient solution, but in the more concentrated Long Ashton nutrient solution (*cf.* Hewitt, 1952, p. 189) which has the following composition:

NUTRIENT SOLUTION 4

Macronutrient elements			Micronutrient elements		
KNO_3	0.202	g/L	$MnSO_4 . 4\ H_2O$	0.00223	g/L
$Ca(NO_3)_2$	0.656	,,	$CuSO_4 . 5\ H_2O$	0.00024	,,
$NaH_2PO_4 . 2H_2O$	0.208	,,	$ZnSO_4 . 7\ H_2O$	0.00029	,,
$MgSO_4 . 7\ H_2O$	0.369	,,	H_3BO_3	0.00186	,,
Ferric citrate	0.0245	,,	$(NH_4)_6Mo_7O_{24} . 4\ H_2O$	0.000035	,,

The nutrient solution in the culture vessels was renewed weekly. The plants were grown in a greenhouse in normal daylight. The maize plants remained in the Long Ashton nutrient solution till they were between 6 and 15 weeks old. The plants were then removed to special nutrient solutions lacking some of the essential mineral requirements. The duration of the pretreatment period varied according to the requirements of the experiments. In the course of this investigation the following special nutrient solutions were employed.

1. *Long Ashton minus N nutrient solution (L.A.–N).* In this solution the normal quantities of $Ca(NO_3)_2$ and KNO_3 were replaced by equimolar quantities of $CaSO_4 . 2H_2O$ and K_2SO_4, respectively.
2. *Long Ashton minus K nutrient solution (L.A.–K).* Here, the amount of KNO_3 was replaced by an equimolar quantity of $NaNO_3$.
3. *Long Ashton minus P nutrient solution (L.A.–P).* In this solution NaH_2PO_4 was replaced by an equimolar quantity of Na_2SO_4.

The respiration rate of the roots was measured with the standard Warburg manometric technique. This technique necessitates the use of excised roots, but it has the advantage of enabling one to measure the oxygen uptake directly. Techniques by which the oxygen consumption is measured in intact roots require much more complicated equipment. With intact roots, the oxygen consumption can be estimated from the carbon dioxide output which can be easily measured by absorption in standard alkali, *e.g.* barium hydroxide (*cf.* Lundegårdh, 1933). However, this method was unsuited to the present investigation because preferential ion absorption gives a variable respiratory quotient (*cf.* Ulrich, 1941).

The Warburg vessels had a volume of about 20 ml. The excised roots were suspended in 3 ml of liquid. In order to absorb the carbon dioxide produced, a roll of Whatman No. 40 starch-free filter paper soaked with 0.2 ml KOH 30 % was inserted in the center well of the respiration vessel. After measuring the basic respiration for 3–4 hours, 0.5 ml salt solution, with or without additional carbohydrate according to the experiment, was tipped in from the side arm. The change in respiration rate during the following period was measured. In some of the experiments, use of the two-vessel method (*cf.* Dixon, 1934 p. 57 and Umbreit *et al.* 1949, p. 17) permitted measurement of the carbon dioxide output as well as the oxygen consumption. The experiments were conducted at 25° C and at a shaking rate of 160–180 cy/min over an arc of 3 cm. The respiration flasks were allowed to equilibrate in

the water thermostat for about 30 minutes. In each respiration vessel approximately 0.57 g fresh weight roots was used, *i.e.* ± 40 mg dry weight, because the root samples had an average dry weight of 7 % of the fresh weight.

§ 3. Analytical methods

In some experiments, the ammonium ion uptake (Table 14, p. 51) and sulfate uptake (p. 39) were measured in addition to respiration. The following quantitative methods of determination were used.

1. *Ammonia*

For the quantitative determination of the ammonium ion content of the nutrient solutions, the micro-Kjeldahl distillation procedure was used. After concluding the respiration experiment, a 2-ml aliquot of the solution containing NH_4^+ was pipetted from the respiration vessel and transferred to the distillation flask. After the addition of excess alkali, the liberated ammonia was distilled off into a known volume of standard acid (10 ml 0.1 N HCl) and the excess acid was back-titrated with standard alkali (0.1 N NaOH).

Later, a boric acid – HCl method for ammonia determination (*cf.* Yuen and Pollard, 1953) was employed. This method has the advantage of rendering the standardization of the boric acid unnecessary, because the distilled ammonia can be titrated directly with the acid. The boric acid acts only as the ammonia absorbent. The distilled ammonia was trapped in 10 ml of 1 % AnalaR boric acid in distilled water and back-titrated with 0.1 N HCl. In a blank titration with the same volume of 1 % boric acid, the amount of acid which was found to produce the same end-point, was subtracted from the values obtained in the analyses. As indicator, an equal volume of 0.2 % alcoholic methyl red and 0.1 % aqueous methylene blue was employed. Duplicate determinations did not differ by more than 1–2 %.

2. *Sulfate*

A colorimetric sulfate determination method using chromate, worked out by Deys and Bosman (personal communication, 1955) was employed. According to their prescription, a 20-ml aliquot of a solution containing 0 – 1.0 m.e. $SO_4^=$/L was pipetted into a volumetric flask of 50 ml capacity. After acidification of the solution with a few drops of 10 % HCl, 10 ml 0.01 N $BaCl_2$ solution was added, mixed well, and the resulting $BaSO_4$ precipitate was allowed to settle for 24 hours. Then, 10 ml of 0.01 N K_2CrO_4 was added. The solution was neutralized against litmus paper with a few drops of NH_4OH, made up to 50 ml and shaken again. After 15 minutes the $BaSO_4$ and $BaCrO_4$ precipitates were removed by centrifugation. The optical density of the clear yellow supernatant solution was measured with a Unicam spectrophotometer at a wave length of 400 mμ. A standard curve of the optical density plotted against sulfate concentration over a range of 0 – 1.0 m.e. $SO_4^=$/L was obtained and used to derive the sulfate concentrations of the unknown solutions. The method was accurate to within 5 %.

§ 4. Experiments

I. *The effect of salts, especially ammonium salts, on the respiration of excised maize roots*

a. The effect of ammonium sulfate on the respiration of newly-emerged primary roots.

The primary roots (radicles) used in these experiments were obtained from 7-day old maize seedlings germinated between humid filter paper sheets on a pad of cotton saturated with distilled water. In this stage of development the roots were still entirely dependent on the food reserve of the seed. Since the seeds were obtained from

exceedingly well-dressed maize plants, the roots had a high carbohydrate and salt content. Therefore, according to the terminology of Hoagland *c.s.*, these roots could be said to be in "high-salt, high-sugar" condition. In order to prevent absorption of previously released salts which could affect the respiration rate before the beginning of the experiment, the distilled water around the roots was renewed daily. It may be emphasized that the experimental conditions were therefore essentially the same as those in the experiments of Lundegårdh, who pretreated his wheat roots with distilled water.

Shortly before the present experiments were started, the maize roots were detached from the seeds and the excised roots thoroughly washed in distilled water. The radicles were then transferred to the Warburg respiration vessels.

In the following experiments, the effect of ammonium salt on the root respiration was compared with that of distilled water (control).

TABLE 5

Respiration rates of excised newly-emerged primary maize roots in response to the addition of ammonium salt. Roots were suspended in distilled water. Respiration rate was measured for 6 consecutive hours. A $(NH_4)_2SO_4$ solution or distilled water (control) was added 3 hours after the start of the experiment. Concentrations given are the final values after salt addition.

Addition	Respiration rate during 6 consecutive hours in cu.mm O_2/hr/mg dr.wt roots					
	1	2	3	4	5	6
H_2O (control)	5.13	5.16	4.81	4.44	4.09	3.94
id.	5.14	4.92	4.45	3.95	3.85	3.44
Mean	5.14	5.04	4.63	4.20	3.97	3.69
%	*111*	*109*	*100*	*91*	*86*	*80*
0.0025 M $(NH_4)_2SO_4$	5.19	4.97	4.63	4.42	3.99	3.94
id.	5.53	4.92	4.45	4.06	4.04	3.67
0.005 M $(NH_4)_2SO_4$	5.23	4.83	4.45	4.29	3.98	3.86
id.	4.83	4.65	4.43	3.91	3.99	3.67
0.01 M $(NH_4)_2SO_4$	4.86	4.65	4.39	4.23	3.70	3.42
id.	4.92	4.75	4.42	3.94	3.98	3.58
0.02 M $(NH_4)_2SO_4$	5.42	5.41	4.90	4.77	4.27	4.07
id.	4.80	4.34	4.00	3.82	3.88	3.23
id.	4.27	3.96	3.76	3.42	3.39	3.08
Mean	5.01	4.72	4.38	4.10	3.91	3.61
%	*114*	*108*	*100*	*94*	*89*	*82*

From Table 5 it appears that the high initial respiration rate decreased rapidly during the 6 consecutive hours of the experiment. The average respiration decrease (*i.e.* the slope of the line) was 0.28 cu. mm O_2 per hour per mg dry weight roots. The addition of $(NH_4)_2SO_4$ had only a negligible effect, because the 2–3 % respiratory increase above control falls inside the experimental error. Therefore, it may be concluded that no "salt effect" can be obtained with these newly-emerged primary roots. This negative result can be explained by the "high-salt" (high-nitrogen) condition of the root tissue involved.

b. The effect of ammonium salts on the root respiration of plants pretreated with dilute calcium sulfate solutions.

LUNDEGÅRDH (1933) stated that in order to obtain a salt-respiration effect the roots should be exposed to distilled water for a certain time previous to the experiment. However, the somewhat longer pretreatment used in the present experiments was found to be injurious to maize roots. Therefore, a pretreatment in very dilute solutions of salts which were not readily taken up was decided upon. For short pretreatments a $CaSO_4$ 10^{-4}M solution, and for longer pretreatments a $CaSO_4$ 10^{-4}M plus KH_2PO_4 10^{-4}M solution, were employed.

In these experiments two different sets of maize plants were used. The plants were grown in a complete L.A. nutrient solution. The plants of Set 1 were 15 weeks old (6/1–22/4.55) and were transferred to the $CaSO_4$ 10^{-4}M solution 2 days previous to the experiment. Set 2 consisted of 17-week old (6/1–5/5.55) plants which were placed on a $CaSO_4$ 10^{-4}M plus KH_2PO_4 10^{-4}M solution 7 days before the experiment.

TABLE 6

Respiration rates of excised roots of maize plants, pretreated with dilute calcium sulfate solutions, in response to ammonium salt addition. Roots were suspended in distilled water. Respiration rate was measured for 7 consecutive hours. A NH_4Cl solution or distilled water (control) was tipped in 3 hours after the start of the experiment. Concentrations are the final values after salt addition.

Addition	Respiration rate during 7 consecutive hours in cu.mm O_2/hr/mg dr.wt roots						
	1	2	3	4	5	6	7
Set 1:							
H_2O (control)	5.24	5.20	4.90	4.46	4.45	4.20	4.04
id.	4.98	4.84	4.83	4.38	4.02	3.89	3.62
Mean	5.11	5.02	4.87	4.42	4.24	4.05	3.83
%	*105*	*103*	*100*	*91*	*87*	*83*	*79*
0.01 M NH_4Cl	5.05	4.78	4.96	5.67	5.68	5.52	5.40
id.	5.90	5.34	5.28	6.11	5.97	5.97	5.67
0.02 M NH_4Cl	6.63	6.59	6.55	6.77	6.73	6.70	6.22
id.	4.48	4.55	4.46	5.36	5.15	5.38	5.22
Mean	5.52	5.32	5.31	5.98	5.88	5.89	5.63
%	*104*	*100*	*100*	*113*	*111*	*111*	*106*
% resp. increase	—	—	—	*22*	*24*	*28*	*27*
Set 2:							
H_2O (control	2.98	2.71	2.60	2.39	2.37	2.32	2.30
%	*115*	*104*	*100*	*92*	*91*	*89*	*88*
0.005 M NH_4Cl	2.86	2.60	2.53	3.16	3.19	2.83	2.82
id.	2.85	2.61	2.53	2.85	2.85	2.61	2.63
0.01 M NH_4Cl	3.99	3.77	3.56	3.73	3.68	3.40	3.38
id.	3.10	3.09	3.12	3.46	3.07	2.95	2.90
0.02 M NH_4Cl	3.60	3.34	3.19	4.10	3.80	3.34	3.38
Mean	3.28	3.08	2.99	3.46	3.32	3.03	3.02
%	*110*	*103*	*100*	*116*	*111*	*101*	*101*
% resp. increase	—	—	—	*24*	*20*	*12*	*13*

Table 6 shows that a 2-day pretreatment in dilute calcium sulfate solution did not essentially affect the rate of root respiration. This is evident from a comparison of the data of Table 6 with Table 5. However, in contrast to this, a pretreatment of 7 days in a $CaSO_4$ 10^{-4}M plus KH_2PO_4 10^{-4}M solution gave a pronounced decrease of the respiration rate. The initial respiration rate of Set 2 was only 60 % of that of Set 1. The decrease in respiration rate, however, was much slower in the roots pretreated for 7 days than in the roots pretreated for 2 days, *i.e.* 0.04 compared to 0.21 cu.mm O_2/hr/mg dr.wt roots, respectively.

In great contrast to the experiments given in Table 5, the addition of ammonium salt initiated a marked (20–28 %) increase in respiration rate compared with the addition of distilled water. Moreover, it can be noted that the respiratory increase showed no correlation with the NH_4^+ concentration applied, since even the lowest NH_4Cl concentration (0.005 M) gave a maximal respiratory response.

Further, from Table 6 it can be observed that although the average respiration rate of roots pretreated for 7 days was much lower than that of roots pretreated for 2 days, the percentage respiratory increase was equal.

The average ammonium ion uptake was about seven times as great as the sulfate ion uptake (analyses not given). Therefore, it seems reasonable to suggest that the respiratory increase is mainly due to ammonium ion uptake.

c. The effect of ammonium salts on the root respiration of plants pretreated with Long Ashton minus N nutrient solution.

The results presented in Section *b* showed that the addition of ammonium salts to roots pretreated in a dilute calcium sulfate solution caused a pronounced increase in respiration rate. The question arises as to whether this respiratory increase is connected with a specific mineral deficiency induced by the pretreatment. If so, a respiratory response could only be initiated by the addition of the particular ion in which the root is deficient. An increase of root respiration due to ammonium salt addition would be more prominent in cases of nitrogen starvation or nitrogen deficiency. To check this hypothesis the following experiments were made.

Maize plants 6 weeks old (26/4–6/6.55) grown on a L.A. solution were transferred to a L.A.-N solution 9 days previous to the experiment.

Table 7 shows that, notwithstanding the pretreatment in the relatively concentrated L.A.-N nutrient solution, the addition of ammonium salt gave a marked ($\pm$ 28 %) increase of respiration above distilled water addition (control). As in the preceding experiments, no correlation between the respiratory increase and the ammonium salt concentrations could be observed, since the lowest $(NH_4)_2SO_4$ concentration used (0.0025 M, *i.e.* 90 p.p.m. NH_4^+) was sufficient to give the maximal respiratory effect. This is not surprising, since, as reported in Chapter II, the NH_4^+ absorption rate increased only negligibly above a NH_4^+ concentration of 10 p.p.m.

TABLE 7

Respiration rates of excised roots of nitrogen-starved maize plants in response to ammonium salt addition. Roots were suspended in distilled water. Respiration rate was measured for 8 consecutive hours. A $(NH_4)_2SO_4$ (NH_4Cl) solution or distilled water (control) was tipped in 3 hours after the start of the experiment. Concentrations given are the final values after salt addition.

Addition	Respiration rate during 8 consecutive hours in cu.mm O_2 /hr /mg dr.wt roots							
	1	2	3	4	5	6	7	8
H_2O (control) %	*84*	*94*	*100*	*91*	*87*	*83*	*79*	*75*
0.0025 M $(NH_4)_2SO_4$	5.27	5.51	5.91	7.38	6.74	6.06	5.59	5.00
id.	4.74	4.93	5.49	6.24	6.04	5.47	5.06	4.73
0.005 M $(NH_4)_2SO_4$	4.16	4.57	4.97	5.75	5.18	4.94	4.30	4.12
id.	5.32	5.57	6.30	7.12	6.77	5.90	5.49	5.02
0.01 M $(NH_4)_2SO_4$	4.73	4.98	5.47	6.39	5.87	5.53	4.93	4.82
id.	4.77	5.06	5.32	6.66	6.39	5.76	5.38	4.89
0.02 M $(NH_4)_2SO_4$	5.47	5.75	6.17	7.96	7.23	6.44	5.89	5.43
id.	4.76	5.13	5.79	6.64	6.25	5.63	5.29	4.75
0.02 M NH_4Cl	5.63	5.69	5.77	7.14	6.14	5.49	5.13	4.87
id.	4.79	5.14	5.62	6.48	6.01	5.49	5.10	4.76
Mean	4.96	5.23	5.68	6.78	6.26	5.67	5.22	4.84
%	*87*	*92*	*100*	*119*	*110*	*100*	*92*	*85*
% resp. increase	—	—	—	*28*	*23*	*17*	*13*	*10*

d. The effect of ammonium sulfate and potassium sulfate on the root respiration of plants pretreated with complete Long Ashton, Long Ashton minus N, or Long Ashton minus K solutions.

In the following experiments the effect of ammonium and potassium salts on the root respiration of plants pretreated with nitrogen- or potassium-free nutrient solutions was investigated. As a control, the same salt additions were made with roots obtained from L.A. grown plants.

All maize plants used in these experiments were of the same batch. They were 13.5 weeks old (18/3–21/6.55) and were put on the mineral-deficiency pretreatments 11 days previous to the experiment.

Table 8 shows that the root respiration rates of the variously pretreated plants were appreciably different. The average respiration rate of L.A., L.A.-N, and L.A.-K roots was 6.68 $\pm$ 0.26, 3.67 $\pm$ 0.27 and 8.07 $\pm$ 0.33 cu.mm O_2/hr/mg dr.wt roots, respectively. The calculated standard errors show that these differences are significant. The root respiration rate of L.A.-N plants was 45 % lower, and that of L.A.-K plants 21 % higher, than that of L.A. plants. The dry weight of pretreated roots did not differ from non-pretreated roots (*i.e.* 7–8 % of the fresh weight), so the respiratory differences cannot be attributed to dry matter differences of the samples.

As can be seen in Table 8, L.A.-N pretreated roots showed a respiratory increase in response to the addition of ammonium sulfate, but an equimolar quantity of potassium sulfate had no effect on the

TABLE 8

Respiration rates of excised roots of maize plants, pretreated with L.A., L.A.-N *and* L.A.-K *nutrient solutions, in response to nitrogen and potassium salt addition.* Roots were suspended in distilled water. Respiration rate was measured for 8 consecutive hours. The salt solution or distilled water (control) was tipped in 3 hours after the start of the experiment. Concentrations given are the final values after salt addition.

Addition	Respiration rate during 8 consecutive hours in cu.mm O_2/hr/mg dr.wt roots.							
	1	2	3	4	5	6	7	8
L.A. Plants:								
H_2O (control)	7.41	6.99	6.84	6.47	6.20	5.60	4.97	4.31
%	*115*	*108*	*106*	*100*	*96*	*87*	*77*	*67*
0.01 M $(NH_4)_2SO_4$ . .	6.46	5.72	5.27	5.01	4.58	4.24	3.40	3.22
0.02 M $(NH_4)_2SO_4$ . .	6.65	6.10	5.78	5.63	5.68	5.02	4.12	3.42
Mean	6.56	5.91	5.53	5.32	5.13	4.63	3.76	3.32
%	*123*	*111*	*104*	*100*	*96*	*87*	*71*	*62*
0.02 M K_2SO_4. . . .	6.20	5.89	5.67	5.59	5.16	4.56	3.97	3.42
%	*111*	*105*	*101*	*100*	*92*	*82*	*71*	*61*
L.A.–N Plants:								
H_2O (control)	3.22	3.23	3.00	2.87	2.57	2.50	2.21	2.05
%	*112*	*113*	*105*	*100*	*90*	*87*	*77*	*71*
0.02 M K_2SO_4. . . .	3.64	3.83	3.57	3.50	3.27	2.98	2.60	2.42
%	*104*	*109*	*102*	*100*	*93*	*85*	*74*	*69*
0.02 M $(NH_4)_2SO_4$ . .	4.14	3.92	3.93	3.64	3.96	3.61	2.87	2.62
%	*114*	*108*	*108*	*100*	*109*	*99*	*79*	*72*
% resp. increase	—	—	—	—	*19*	*12*	*2*	*1*
L.A.–K Plants:								
H_2O (control)	7.66	6.89	6.10	5.65	5.33	4.87	4.00	3.43
%	*136*	*122*	*108*	*100*	*94*	*86*	*71*	*61*
0.01 M $(NH_4)_2SO_4$ . .	7.15	6.24	5.70	5.57	5.21	4.82	4.18	3.57
0.02 M $(NH_4)_2SO_4$ . .	9.00	8.17	6.67	5.94	5.62	4.93	3.98	3.26
Mean	8.08	7.21	6.19	5.76	5.42	4.88	4.08	3.42
%	*140*	*125*	*107*	*100*	*94*	*85*	*71*	*59*
0.01 M K_2SO_4. . . .	7.91	6.81	6.23	5.66	5.12	4.46	3.73	3.12
0.02 M K_2SO_4. . . .	8.63	7.93	6.60	5.65	4.86	4.13	3.29	2.65
Mean	8.27	7.37	6.42	5.66	4.99	4.30	3.51	2.89
%	*146*	*130*	*113*	*100*	*88*	*76*	*62*	*51*
% resp. decrease	—	—	—	—	*6*	*10*	*9*	*10*

respiration. Thus, in this case (see p. 39) it is evident that ammonium ions rather than sulfate ions are responsible for the respiratory increase. This conclusion is contradictory to the "anion respiration" hypothesis of Lundegårdh.

Summarizing the conclusions suggested by the results presented in Table 8, it can be said that:

(i) in *L.A. plants*, the addition of $(NH_4)_2SO_4$ to the roots did not affect the respiration rate. The addition of K_2SO_4, however, caused a 4–6 % decrease in respiration rate.

(ii) in *L.A.-N plants*, the addition of $(NH_4)_2SO_4$ stimulated the root respiration rate by about 20 %, whereas the addition of K_2SO_4 had no effect.

(iii) in *L.A.-K plants*, the addition of K_2SO_4 gave an appreciable (6–12 %) depression of the increased respiration rate caused by potassium starvation. This effect must be due solely to potassium ion uptake, because an equimolar amount of ammonium sulfate did not affect the respiration rate of potassium-starved roots.

From the above facts, it can be concluded that nitrogen and potassium salts have opposite effects on the respiration rate. Nitrogen starvation decreases the respiration rate very markedly, whereas potassium starvation increases it. The addition of nitrogen salt increases the root respiration of nitrogen-starved plants and the addition of potassium salt decreases the root respiration of potassium-starved plants.

e. The effect of various salts on root respiration of plants pretreated with complete Long Ashton solution or with nitrogen-free Long Ashton solution.

In all the above experiments where a salt-respiration effect was noted, the roots had been suspended in *distilled water* for 3–4 hours before salt addition. It is possible that this exposure to distilled water may have induced a low salt condition in the roots. Therefore, the respiration increase may have been due not only to the particular pretreatment (*e.g.* nitrogen starvation), but also to the distilled water pretreatment.

As a matter of fact, LUNDEGÅRDH (1933) stated that a distilled water pretreatment is indispensable for obtaining a salt-respiration effect. This can be verified by measuring the respiration before salt addition in a nutrient solution which lacks only one particular ion, instead of measuring it in the distilled water. In this way a respiratory effect induced specifically by ammonium salt addition to nitrogen-deficient roots suspended in L.A.-N solution can be studied.

Moreover, the evidence of a relation between respiratory stimulation caused by ammonium salt addition and nitrogen starvation will be greatly strengthened if other nitrogen-containing salts can be shown to produce a similar respiratory response in nitrogen-deficient roots. The effect of nitrates was investigated, and for the sake of comparison (controls) various other salts such as NaCl, Na_2SO_4 and K_2SO_4 were included in the experiments.

Two sets of plants of different ages were used. Set 1 was 8.5 weeks old (26/4–25/6.55) and was divided into two groups: one of these was pretreated for 3 weeks on L.A.–N solution and the other remained on L.A. solution. Set 2 consisted of 18-week old (18/3–22/7.55) plants of which the L.A.–N group was starved for 6 weeks on a nitrogen-free L.A. solution.

From the results presented in Table 9, it is clear that distilled-water pretreatment is not a requisite for obtaining a salt-respiration effect. Although before salt addition many ions were already available to the roots, a respiratory increase took place only after the addition of the ion in which the root was deficient. Therefore, a causal relation between respiratory response and the particular salt deficiency seems to be incontrovertible.

The following detailed conclusions can be derived from Table 9.

(i) *L.A. roots* of 8.5-week old plants show a much higher respiration rate than those of 18-week old plants, *i.e.* 7.35 against 4.35 cu.mm O_2/hr/mg dr.wt roots.

(ii) A prolonged treatment with L.A.-N solution decreased the root respiration rate appreciably. The root respiration of plants pretreated for 4 and 6 weeks was only 50 % and 44 %, respectively, of that of L.A. plants.

(iii) In *L.A. plants*, the root respiration is hardly increased (2–5 %) by the addition of $(NH_4)_2SO_4$. The addition of $NaNO_3$ and NaCl gave no increase in respiration rate. The addition of K_2SO_4 decreased the respiration rate by 5–12 %.

(iv) In *L.A.-N roots*, the addition of $(NH_4)_2SO_4$ produced a respiratory increase of 10–15 %. The addition of $NaNO_3$ also produced an increase of respiration rate, but the response was more gradual than in the case of ammonium salt addition. The maximum respiratory increase (33 %) was not reached until three hours after nitrate addition. This retarded increase can be understood, since nitrate ions are absorbed by maize roots at a much slower rate than ammonium ions and the nitrate ion has to be reduced before transformation into organic nitrogen compounds. However, the ultimately greater respiratory increase due to nitrates (33 %) compared to that due to ammonium salt (15 %) showed that for maize nitrate nitrogen is a more suitable nitrogen source than ammonium nitrogen. This is supported by experiments with intact maize plants, where ammonium nutrition gave a steady decline in the ammonium-absorbing capacity of the roots (Chapter II, § 8, V).

The addition of Na_2SO_4 had no effect on the respiration rate. However, in contrast to the L.A. roots, L.A.-N roots showed a small respiratory increase (5–9 %) in response to NaCl addition. No immediate explanation was found for this phenomenon. It should be noted that neither the L.A. nor the L.A.-N solution contained chloride ions.

TABLE 9

Respiration rates of excised maize roots, pretreated with Long Ashton and Long Ashton minus N nutrient solutions, in response to the addition of various salts. Roots were suspended in Long Ashton and Long Ashton minus N solutions. Respiration was measured for 7 consecutive hours. Salt solutions and L.A. or L.A.-N solutions (controls) were added 3 hours after the start of the experiment. Concentrations are the final values after salt addition.

Set	Addition	Respiration during 7 consecutive hours in cu.mm O_2/hr/mg dr.wt roots						
		1	2	3	4	5	6	7
	L.A. Plants:							
1	L.A. (control)	7.66	7.04	6.59	6.36	6.00	5.58	5.12
	%	*116*	*107*	*100*	*97*	*91*	*85*	*78*
	0.005 M $(NH_4)_2SO_4$	7.86	7.19	6.49	6.27	5.98	5.48	5.17
	0.01 M $(NH_4)_2SO_4$	7.01	6.72	6.45	6.85	6.21	5.68	5.59
1	id.	6.94	6.78	6.32	6.50	5.92	5.66	5.41
	0.02 M $(NH_4)_2SO_4$	7.66	7.94	6.30	5.90	5.59	5.23	4.80
	id.	6.94	6.46	5.96	6.22	5.64	5.19	5.03
	Mean	7.28	7.02	6.30	6.35	5.87	5.45	5.20
	%	*116*	*111*	*100*	*101*	*93*	*87*	*83*
	% resp. increase	—	—	—	*4*	*2*	*2*	*5*
1	0.01 M $NaNO_3$	4.16	3.74	3.53	3.32	3.28	3.19	3.00
	0.02 M $NaNO_3$	4.55	4.05	3.91	3.73	3.63	3.51	3.11
	Mean	4.36	3.90	3.72	3.53	3.46	3.35	3.06
	%	*117*	*105*	*100*	*95*	*93*	*90*	*82*
2	0.01 M NaCl	3.79	3.41	3.27	3.00	3.01	2.81	2.62
	0.02 M NaCl	3.00	2.80	2.68	2.49	2.42	2.33	2.20
	Mean	3.40	3.11	2.98	2.75	2.72	2.57	2.41
	%	*114*	*104*	*100*	*92*	*91*	*86*	*81*
2	0.01 M K_2SO_4	5.56	5.31	5.10	4.30	4.18	3.95	3.73
	0.02 M K_2SO_4	5.05	4.79	4.35	3.78	3.59	3.40	3.14
	Mean	5.31	5.05	4.73	4.04	3.89	3.68	3.44
	%	*112*	*107*	*100*	*85*	*82*	*78*	*73*
	% resp. decrease	—	—	—	*12*	*9*	*7*	*5*

TABLE 9 (*continued*)

Set	Addition	Respiration during 7 consecutive hours in cu.mm O_2/hr/mg dr.wt roots						
		1	2	3	4	5	6	7
	L.A.–N Plants:							
1	L.A.–N (control) . . .	3.10	3.13	3.21	3.34	3.19	2.97	2.84
	%	*97*	*98*	*100*	*104*	*99*	*93*	*88*
	0.005 M $(NH_4)_2SO_4$. .	3.60	3.62	3.46	4.07	4.02	3.79	3.46
	0.01 M $(NH_4)_2SO_4$. .	3.86	3.51	3.46	4.19	4.27	3.88	3.70
1	id.	4.02	4.01	3.89	4.42	4.41	3.94	3.75
	0.02 M $(NH_4)_2SO_4$. .	4.42	4.58	4.56	4.77	4.58	4.26	3.96
	id.	3.14	3.27	3.11	3.85	3.83	3.46	3.27
	Mean	3.81	3.80	3.70	4.26	4.22	3.87	3.63
	%	*103*	*103*	*100*	*115*	*114*	*105*	*98*
	% resp. increase	—	—	—	*11*	*15*	*12*	*10*
2	L.A.–N (control) . . .	1.41	1.42	1.45	1.41	1.30	1.26	1.30
	%	*97*	*98*	*100*	*97*	*90*	*87*	*90*
	0.01 M $NaNO_3$. . . .	1.92	1.93	1.91	2.03	2.37	2.39	2.23
	0.02 M $NaNO_3$. . . .	2.42	2.34	2.30	2.46	2.81	2.82	2.60
2	id.	2.40	2.36	2.45	2.49	2.80	2.79	2.68
	id.	1.48	1.41	1.30	1.33	1.63	1.54	1.51
	Mean	2.06	2.01	1.99	2.08	2.40	2.39	2.26
	%	*104*	*101*	*100*	*105*	*121*	*120*	*114*
	% resp. increase	—	—	—	*8*	*31*	*33*	*24*
	0.01 M Na_2SO_4. . . .	1.55	1.60	1.61	1.50	1.48	1.28	1.30
2	0.02 M Na_2SO_4. . . .	1.60	1.43	1.30	1.25	1.27	1.12	1.11
	id.	1.88	1.86	1.74	1.58	1.59	1.53	1.37
	Mean	1.68	1.63	1.55	1.44	1.45	1.31	1.26
	%	*108*	*105*	*100*	*93*	*94*	*85*	*81*
	0.02 M NaCl.	1.99	2.03	2.05	2.15	2.10	1.95	1.98
2	0.01 M NaCl.	2.55	2.55	2.53	2.53	2.41	2.29	2.19
	Mean	2.27	2.29	2.29	2.34	2.26	2.12	2.09
	%	*99*	*100*	*100*	*102*	*99*	*93*	*91*
	% resp. increase	—	—	—	*5*	*9*	*6*	*1*

II. *The effect of sugars, with or without simultaneous addition of ammonium salts or nitrates, on the root respiration and the determination of the respiratory quotient in normal and in nitrogen-starved maize roots*

In these experiments two sets of maize plants differing in age as well in pretreatment period were used. Set 1 consisted of plants 11 weeks old (26/4–14/7.55) of which one group was nitrogen-starved for 6.5 weeks. In these plants the effect of the additions of L.A., L.A.-N, $(NH_4)_2SO_4$, 2 % sucrose, and 2 % sucrose plus $(NH_4)_2SO_4$ on the root-respiration rate was studied (Table 10). Set 2 included plants 12 weeks old (27/5–22/8.55) which were nitrogen-starved on a L.A.-N solution for 3.5 weeks previous to the experiment. In these L.A.-N plants the effect of additions of 2 % glucose, 2 % glucose plus $(NH_4)_2SO_4$, and 2 % glucose plus $NaNO_3$ on the root respiration was investigated (Table 11). The last experiment presented in Table 11 dealt with roots obtained from a full-grown maize plant (Chapter II, § 3) grown for about 5 weeks on a Woodford and Gregory nutrient solution.

TABLE 10

Root respiration rates of normal and nitrogen-starved maize plants, in response to the additions of Long Ashton, Long Ashton minus N, ammonium sulfate, 2 % sucrose, and 2 % sucrose + ammonium sulfate. Excised roots of the normal and nitrogen-starved maize plants were suspended in L.A. or L.A.-N nutrient solution. Respiration was measured for 7 consecutive hours. Substrates were added 3 hours after the start of the experiment. Salt and sugar concentrations are the final values after addition.

Addition	Respiration during 7 consecutive hours in cu.mm O_2/hr/mg dr.wt roots						
	1	2	3	4	5	6	7
L.A. Plants							
L.A. (control.)	6.29	6.13	5.98	5.61	5.00	4.70	4.24
%	*105*	*103*	*100*	*94*	*84*	*79*	*71*
0.01 M $(NH_4)_2SO_4$	5.87	5.58	5.20	5.23	4.62	4.53	4.25
0.02 M $(NH_4)_2SO_4$	6.36	5.90	5.56	5.44	5.15	4.62	4.29
Mean	6.12	5.74	5.38	5.34	4.89	4.58	4.27
%	*114*	*107*	*100*	*99*	*91*	*85*	*79*
% resp. increase	—	—	—	*5*	*7*	*6*	*8*
2% sucrose	5.05	4.21	4.02	4.89	5.20	5.44	5.47
%	*126*	*105*	*100*	*122*	*129*	*135*	*136*
% resp. increase	—	—	—	*28*	*45*	*56*	*65*
2% sucrose + 0.005 M $(NH_4)_2SO_4$	6.36	5.78	5.35	6.46	7.00	6.84	6.34
2% sucrose + 0.01 M $(NH_4)_2SO_4$	4.96	4.68	4.29	5.25	5.51	5.89	5.53
Mean	5.66	5.23	4.82	5.86	6.26	6.37	5.94
%	*117*	*109*	*100*	*122*	*130*	*132*	*123*
% resp. increase	—	—	—	*28*	*46*	*53*	*52*
L.A.–N Plants							
L.A.–N. (control)	1.66	1.62	1.50	1.70	1.65	1.63	1.57
%	*111*	*108*	*100*	*113*	*110*	*109*	*105*
0.01 M $(NH_4)_2SO_4$	1.99	1.88	1.88	2.42	2.26	2.25	2.16
0.02 M $(NH_4)_2SO_4$	1.99	1.77	1.73	2.26	2.38	2.17	2.15
Mean	1.99	1.83	1.81	2.34	2.32	2.21	2.16
%	*110*	*101*	*100*	*129*	*128*	*122*	*119*
% resp. increase	—	—	—	*16*	*18*	*13*	*14*
2% sucrose	1.69	1.53	1.81	1.96	2.18	2.26	2.29
%	*93*	*85*	*100*	*108*	*120*	*125*	*127*
% resp. increase	—	—	—	*–5*	*10*	*16*	*22*
2% sucrose + 0.005 M $(NH_4)_2SO_4$	1.92	1.76	1.69	2.58	3.04	3.10	3.18
2% sucrose + 0.01 M $(NH_4)_2SO_4$	1.60	1.51	1.54	2.23	2.62	2.71	2.85
Mean	1.76	1.64	1.62	2.41	2.83	2.91	3.02
%	*109*	*101*	*100*	*149*	*175*	*180*	*186*
% resp. increase	—	—	—	*36*	*65*	*71*	*81*

From the results collected in Tables 10 and 11 the following facts appear:

(i) In *L.A. roots* (Table 10), the addition of $(NH_4)_2SO_4$ gave only a small increase in respiration rate (5–8 %). This effect was probably caused by the still great NH_4^+–

TABLE 11

Root respiration rates of nitrogen-starved maize plants in response to the additions of 2 % glucose, 2 % glucose + ammonium sulfate, and 2 % glucose + sodium nitrate. Excised roots were suspended in L.A.-N nutrient solution. Respiration was measured for 13 consecutive hours. Substrates were added 3 hours after the start of the experiment. Sugar and salt concentration are the final values after addition.

Addition		Respiration during 13 consecutive hours in cu.mm O_2 and CO_2/hr/mg dr.wt roots												
		1	2	3	4	5	6	7	8	9	10	11	12	13
2% glucose	O_2	1.68	1.62	1.53	1.98	1.84	2.15	2.16	2.16	2.14	2.17	2.12	—	—
	CO_2	1.65	1.62	1.51	1.89	1.79	2.17	2.15	2.17	2.10	2.09	2.05	—	—
	R.Q.	0.98	1.00	0.99	0.95	0.97	1.01	1.00	1.00	0.98	0.96	0.97	—	—
	O_2%	*110*	*106*	*100*	*129*	*120*	*141*	*141*	*141*	*140*	*142*	*139*	—	—
2% glucose + 0.01 M $(NH_4)_2SO_4$	O_2	1.82	1.58	1.53	2.41	2.73	2.79	2.82	2.83	2.86	2.99	3.08	3.00	3.22
	CO_2	1.88	1.58	1.55	2.53	2.70	2.81	2.81	2.84	2.81	2.95	3.05	2.90	3.23
	R.Q.	1.03	1.00	1.01	1.05	0.99	1.01	1.00	1.00	0.98	0.99	0.99	0.97	1.00
2% glucose + 0.02 M $(NH_4)_2SO_4$	O_2	1.71	1.55	1.48	2.35	2.74	2.85	2.77	2.82	2.94	3.00	3.03	3.06	3.14
	CO_2	1.71	1.63	1.55	2.49	2.84	2.96	2.85	2.91	3.02	3.05	3.10	2.82	3.28
	R.Q.	1.00	1.05	1.05	1.06	1.04	1.04	1.03	1.03	1.03	1.02	1.02	0.92	1.04
	O_2 mean	1.77	1.57	1.51	2.38	2.74	2.82	2.80	2.83	2.90	3.00	3.06	3.03	3.18
	O_2%	*117*	*104*	*100*	*158*	*181*	*187*	*185*	*187*	*192*	*199*	*203*	*201*	*211*
2% glucose + 0.01 M $NaNO_3$	O_2	1.96	1.86	1.79	2.36	2.90	3.08	3.26	3.44	3.55	3.59	3.79	3.91	4.05
	CO_2	1.76	1.81	1.57	2.44	3.07	3.38	3.62	4.01	3.95	4.32	4.56	4.80	5.06
	R.Q.	0.90	0.97	0.88	1.03	1.06	1.10	1.11	1.17	1.11	1.20	1.20	1.23	1.25
2% glucose + 0.02 M $NaNO_3$	O_2	2.00	1.84	1.68	2.63	3.03	3.17	3.42	3.60	3.71	3.81	3.95	4.11	4.23
	CO_2	1.81	1.69	1.48	2.35	3.16	3.56	4.00	4.22	4.36	4.59	4.71	4.99	5.19
	R.Q.	0.91	0.92	0.88	0.89	1.04	1.12	1.17	1.17	1.18	1.20	1.19	1.21	1.23
	O_2 mean	1.98	1.85	1.74	2.50	2.97	3.13	3.34	3.52	3.63	3.70	3.87	4.01	4.14
	O_2%	*114*	*106*	*100*	*144*	*171*	*180*	*192*	*202*	*209*	*213*	*222*	*230*	*238*
2% glucose + 0.02 M $NaNO_3$	O_2	2.82	2.57	2.37	3.65	4.20	4.10	4.12	4.23	4.37	4.48	4.58	4.62	4.73
	CO_2	2.51	2.29	2.20	3.57	4.53	4.59	4.62	4.90	4.99	5.31	5.50	5.66	5.77
	R.Q.	0.89	0.89	0.93	0.98	1.08	1.12	1.12	1.16	1.14	1.19	1.20	1.23	1.22

absorption capacity of these L.A. roots. The addition of sucrose alone produced a very marked (± 45–65 %) respiratory increase. However, the respiratory increase due to the addition of 2 % sucrose plus $(NH_4)_2SO_4$ was of about the same magnitude (46–53 %). From these observations, it can be concluded that these L.A. roots require carbohydrate rather than ammonium nitrogen to produce a respiratory increase.

(ii) In *L.A.-N roots* (Table 10), the respiration rate is only 31 % of that of L.A. roots. The addition of $(NH_4)_2SO_4$ to these roots gave a respiratory increase of 13 18 %. The addition of sucrose alone increased the respiration only 20 %. However, the addition of sucrose plus $(NH_4)_2SO_4$ produced, in the first hour, a respiratory increase of 36 %, which rose to about 80 % after four hours.

(iii) In the *L.A.-N roots* of Set 2 (Table 11), the decrease in respiration rate during the experimental period was not measured. Therefore, the response was compared to the respiration rate in the 3rd hour, *i.e.* at the time of substrate addition. The effect of glucose alone gave a more marked respiratory increase in these L.A.-N roots than the sucrose addition in the previous set (Table 10), because in the third hour after sugar addition a increase of 40 % could already be noted. This increase remained more or less constant till the end of the experiment.

(iv) In these *L.A.-N roots* (Table 11), the addition of 2 % glucose plus $NaNO_3$ at first produced only a rather small respiratory increase compared to the addition of 2 % glucose plus $(NH_4)_2SO_4$. However, between the third and fourth hour after substrate addition this situation changed, and the respiratory increase due to nitrate addition became greater than that due to ammonium salt addition. Ten hours after substrate addition the respiratory increase was as high as 140 %, *i.e.* 30 % greater than the respiratory increase due to ammonium salt addition for the same length of time.

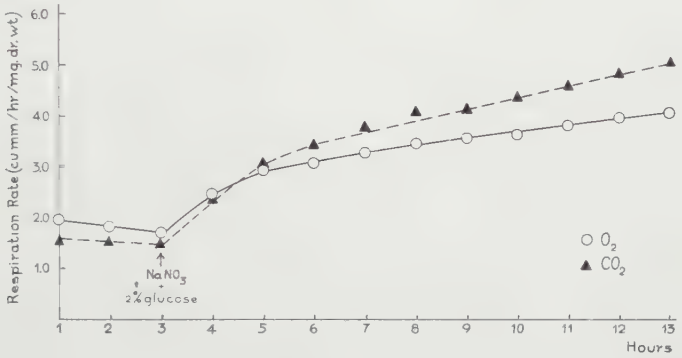


Fig. 5. The effect of $NaNO_3$ plus 2 % glucose on the respiration rate of excised roots of nitrogen-starved maize plants.

(v) The respiratory quotient (R.Q.) of the root respiration changed as a consequence of the addition of glucose plus $NaNO_3$ and glucose plus $(NH_4)_2SO_4$ (Table 11). Glucose addition by itself did not appreciably affect the initial R.Q. value, as can be seen from the figure of 0.99 in the 3rd hour (previous to sugar addition) and of 0.97 in the 11th hour (8 hours after sugar addition). Nitrate addition produced a marked increase of the R.Q.; previous to its addition the R.Q. = 0.88, while ten hours after nitrate plus sugar addition the R.Q. = 1.23–1.25 (Fig. 5). This increase of the R.Q. can be explained by the fact that the oxygen of the nitrate serves as a hydrogen acceptor and substitutes for a portion of the oxygen which would otherwise be consumed (*cf.* BONNER, 1950). In contrast to this, the ammonium salt addition tended to decrease the R.Q. value, *e.g.* 1.01–1.05 before addition compared to 0.92–0.97 nine hours after ammonium salt plus sugar addition.

(vi) The roots of the full-grown maize plant grown in a Woodford and Gregory solution (Table 11, last experiment) produced, in response to nitrate plus sugar addition, the same respiratory increase as roots of nitrogen-starved maize plants. Therefore, it can be concluded that these full-grown maize plants had become to some degree nitrogen-deficient on this Woodford and Gregory solution.

III. *The effect of NH_4^+-bearing ion exchange resins on the root respiration of nitrogen-starved maize plants*

LUNDEGÅRDH (1933) suggested in his "anion respiration" hypothesis that respiratory increase is linked to the absorption of anions only. In the experiments collected in Table 8, it was demonstrated that the addition of $(NH_4)_2SO_4$ produced a respiratory increase, but that the addition of a equimolar amount of K_2SO_4 did not effect the respiration. This result indicates that NH_4^+ ions alone are responsible for this respiratory increase. Because this conclusion is at variance with Lundegårdh's "anion respiration" theory, a more direct proof was desirable.

Synthetic ion exchangers, bearing exchangeable mineral ions, offer

a means of exposing plant roots to absorbable ions in the absence of absorbable ions of opposite sign. For the same purpose, JENNY and COWAN (1933) used natural clays in an experiment on the calcium-hydrogen ion exchange in soya bean plants (Chapter I, § 2). Recently, EPSTEIN (1954) used the synthetic ion exchange resins XE-97 and Dowex 50 to perform experiments on the respiration of excised barley roots in response to the addition of the potassium or calcium form of these resins.

In the present study, the synthetic cation exchangers Amberlite IR-120 and Amberlite IRC-50 were used. Amberlite IR-120 is a strong-acid exchanger which consists of cross-linked polystyrene with the functional -SO_2OH group, whereas Amberlite IRC-50 is a weak-acid exchanger composed of cross-linked methacrylic acid with the functional -COOH group. All resins used were of Analytical Grade.

TABLE 12

Respiration rates of excised roots of maize plants, pretreated with nitrogen-free Long Ashton solution, in response to the addition of NH_4^+, Na^+ *or* H^+ *form of Amberlite* IR-120 *ion exchange resin.* Roots were suspended in distilled water. Respiration rate was measured for 8 consecutive hours. Resin was added 3 hours after the start of the experiment. Concentrations apply to the final suspensions after resin addition.

Set	Addition	Respiration rate during 7 consecutive hours in cu.mm O_2/hr/mg dr.wt roots						
		1	2	3	4	5	6	7
	Na^+form (control)							
3	100 m.e.Na^+/L	5.65	5.61	5.46	5.30	5.26	4.99	5.24
4	150 m.e. ,, ...	1.56	1.71	1.76	1.37	1.67	1.54	1.56
	Mean	3.61	3.66	3.61	3.34	3.47	3.27	3.40
	%	*100*	*101*	*100*	*93*	*96*	*91*	*94*
	NH_4^+form							
1	50 m.e. NH^+_4/L	4.53	4.48	4.33	4.49	4.20	3.99	3.64
1	100 m.e. ,, ...	4.18	4.18	4.00	4.36	4.31	4.26	4.21
1	150 m.e. ,, ...	3.84	3.88	3.53	4.21	3.94	3.93	3.62
2	50 m.e. ,, ...	2.61	2.48	2.56	3.05	2.69	2.70	2.50
2	100 m.e. ,, ...	2.42	2.38	2.34	2.75	2.53	2.61	2.47
2	200 m.e. ,, ...	2.49	2.32	2.37	2.92	2.72	2.68	2.64
2	300 m.e. ,, ...	1.90	1.88	1.88	2.51	2.20	2.27	2.25
2	400 m.e. ,, ...	2.29	2.27	2.25	2.95	2.61	2.65	2.53
2	500 m.e. ,, ...	2.65	2.60	2.54	3.14	3.01	2.92	2.96
3	100 m.e. ,, ...	5.03	4.80	4.64	5.49	5.28	5.07	5.02
4	150 m.e. ,, ...	2.14	2.17	2.18	2.55	2.58	2.39	2.44
	Mean	3.10	3.04	2.97	3.49	3.28	3.22	3.12
	%	*104*	*102*	*100*	*118*	*110*	*108*	*105*
	% resp. increase	—	—	—	*25*	*14*	*17*	*11*
	H^+ form							
1	50 m.e. H^+/L	3.94	3.98	3.78	3.34	3.15	3.03	2.77
1	100 m.e. ,, ...	4.06	4.18	4.02	3.31	3.11	3.04	2.82
1	150 m.e. ,, ...	3.99	3.96	3.74	3.18	3.00	2.84	2.68
	Mean	4.00	4.04	3.85	3.28	3.09	2.97	2.76
	%	*104*	*105*	*100*	*85*	*80*	*77*	*72*
	% resp. decrease	—	—	—	*8*	*16*	*14*	*22*

TABLE 13

Respiration rates of excised roots of maize plants, pretreated with nitrogen-free Long Ashton solution, in response to the addition of NH_4^+, Na^+ *or* H^+*form of Amberlite* IRC-50 *ion exchange resin.* Roots were suspended in distilled water. Respiration rate was measured for 8 consecutive hours. Resin was added 3 hours after the start of the experiment. Concentrations apply to the final suspensions after resin addition.

Set	Addition	Respiration rate during 7 consecutive hours in cu.mm O_2/hr/mg dr.wt roots						
		1	2	3	4	5	6	7
	Na^+form (control)							
4	100 m.e. Na^+/L	2.05	2.18	1.98	1.91	1.86	1.79	1.55
4	150 m.e. ,, ...	2.36	2.19	2.23	2.05	1.99	1.90	1.68
	Mean	2.21	2.19	2.11	1.98	1.93	1.85	1.62
	%	*105*	*104*	*100*	*94*	*91*	*88*	*77*
	NH_4^+form							
1	50 m.e. NH_4^+/L	4.32	4.05	3.74	4.14	3.97	3.71	3.74
1	100 m.e. ,, ...	4.67	4.46	4.24	4.77	4.58	4.45	4.29
1	150 m.e. ,, ...	4.09	3.80	3.43	3.99	3.75	3.64	3.60
2	50 m.e. ,, ...	2.53	2.29	2.08	2.46	2.38	2.24	2.27
2	100 m.e. ,, ...	3.12	2.84	2.72	3.34	3.08	2.98	2.89
2	200 m.e. ,, ...	2.15	1.95	1.88	2.62	2.29	2.14	2.12
2	300 m.e. ,, ...	2.37	2.40	2.15	3.03	2.67	2.55	2.58
2	400 m.e. ,, ...	2.13	2.03	1.85	2.66	2.36	2.25	2.23
2	500 m.e. ,, ...	2.67	2.62	2.39	3.42	3.13	2.86	2.80
4	100 m.e. ,, ...	2.39	2.64	2.57	3.00	2.98	2.72	2.60
4	150 m.e. ,, ...	2.57	2.51	2.42	3.05	2.88	2.69	2.56
	Mean	3.00	2.87	2.68	3.32	3.10	2.93	2.88
	%	*112*	*107*	*100*	*124*	*116*	*109*	*107*
	% resp. increase	—	—	—	*30*	*25*	*21*	*30*
	H^+form							
1	50 m.e. H^+/L	3.22	3.29	3.03	3.06	2.84	2.72	2.49
1	100 m.e. ,, ...	3.49	3.45	3.30	3.14	2.89	2.75	2.55
1	150 m.e. ,, ...	4.13	4.02	3.67	3.69	3.45	3.13	2.97
	Mean	3.61	3.59	3.33	3.30	3.06	2.87	2.67
	%	*108*	*108*	*100*	*99*	*92*	*86*	*80*
	% resp. decrease	—	—	—	*–5*	*–1*	*2*	*–3*

The NH_4^+ and Na^+ forms of each resin were prepared by percolating the H^+form in a column with 1 N NH_4OH or 1 N NaOH. The resins were then washed thoroughly to remove the excess NH_4^+ or Na^+ ions, dried, and ground to powder in a porcelain ball mill. The powdered resin was passed through a copper sieve with a mesh width of about 60 μ. The sifted resin was put into the side arm of the respiration flask and tipped in after the basic respiration rate had been measured for 3 hours.

The NH_4^+ content of the resins was measured with the micro Kjeldahl and boric acid procedure described above (§ 2). Amberlite IR-120 and Amberlite IRC-50 whensaturated with NH_4^+ ions contained 3.5 and 2.5 m.e. NH_4^+ per g dry weight resin, respectively. The various NH_4^+ concentrations administered in the experiments were obtained by adding accurately weighed amounts of resin to the distilled water in the respiration flask.

In the experiments described below, the effect of the NH_4^+, Na^+, and H^+form of these two resins on the respiration of nitrogen-starved maize roots was studied. The H^+forms of these resins showed a low pH in distilled water. For Amberlite IR-120 this pH = 2.5–3.0, whereas a suspension of Amberlite IRC-50 had a pH = ± 4.0. When thoroughly washed, the NH_4^+ and Na^+ saturated forms had a pH = 6.0–6.5.

In these experiments four different sets of maize plants were used. Sets 1, 2, 3 and 4 consisted of maize plants aged 10.5 (27/5–10/8.55), 11.5 (27/5–17/8.55), 12 (22/6–16/9.55) and 16 (27/5–16/9.55) weeks, respectively. In the same sequence, these sets of plants were treated with a L.A.-N solution for 12, 19, 26 and 49 days previous to the experiment.

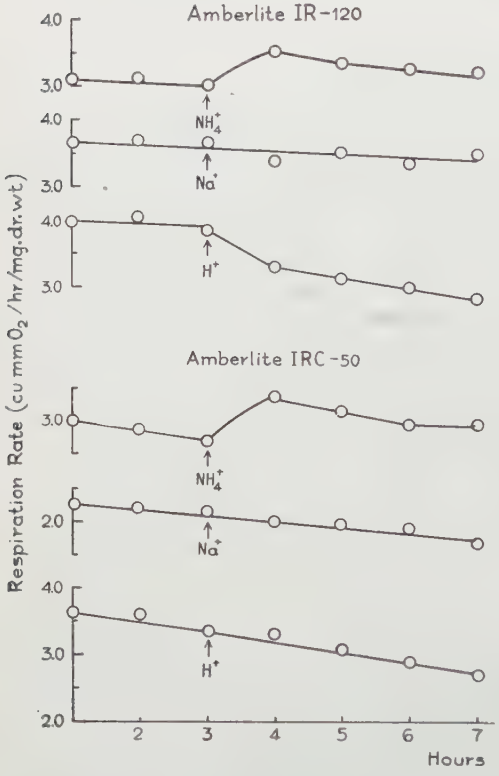


Fig. 6.
The effect of the addition of NH_4^+, Na^+, or H^+form of Amberlite IR-120 and Amberlite IRC-50 ion exchange resins on the root respiration rate of excised roots of nitrogen-starved maize plants.

From the results in Tables 12 and 13 (see also Fig. 6), it is clear that the addition of NH_4^+–bearing resin produced a marked respiratory increase. Because the resin suspensions used may be called one-ion NH_4^+ solutions, the respiratory response can only be due to NH_4^+ion uptake. Hence, according to the terminology of LUNDEGÅRDH (1933), we can speak of a "cation respiration".

In Amberlite IR-120, the NH_4^+form produced a respiratory increase

of 11–25 % above the rate after the addition of the Na^+form (control), whereas the H^+form gave a respiratory decrease of 8–22 %. In Amberlite IRC-50, the addition of the NH_4^+form gave 21–30 % respiratory increase compared to the response after the addition of the Na^+form (control). The addition of the H^+form of the latter resin produced a decrease of 1–2 %. The respiratory decrease observed in response to the addition of the H^+form of these resins may be caused by an injurious effect of low pH on the root. Therefore, it is understandable that this decrease was much more pronounced after the addition of the H^+form of the strong-acid exchanger Amberlite IR-120 than after the addition of the H^+form of the weak-acid exchanger Amberlite IRC-50. The respiratory increase in response to the addition of the NH_4^+ form is lower in Amberlite IR-120 than in Amberlite IRC-50. This effect may be caused by the fact that the adsorbed NH_4^+ ions have a lower exchangeability in the strong-acid exchanger than in the weak-acid exchanger. In a special experiment with NH_4^+–bearing ion exchangers, measurements were made both of the amount of absorbed NH_4^+ ions and the amount of oxygen consumed above the control level.

These results are presented in Table 14.

TABLE 14

Ratio between respiratory increase above control level after resin addition and the amount of ammonium ions absorbed in 4-hour experiments with NH_4^+*–bearing ion exchange resins.* Roots were suspended in distilled water.

Resin	per mg dr.wt roots			ratio
	respiratory increase above control level		NH_4^+ uptake in μ.e.	$\frac{NH_4^+ \text{ uptake}}{\text{Resp. increase}}$
	in μL O_2	in μ.e. O_2		
Amberlite IR–120				
100 m.e. NH_4^+/L	3.48	0.155	0.754	4.9
150 m.e. ,,	1.81	0.081	0.615	7.6
Amberlite IRC–50				
100 m.e. NH_4^+/L	2.31	0.103	0.635	6.2
150 m.e. ,,	2.73	0.122	0.605	5.0
Mean	2.58	0.115	0.652	5.9

Table 14 shows that the ratio between NH_4^+ion absorption and extra O_2 consumption varies from 4.9 to 7.6 with an average of 5.9. Syrett (1953 a, b) found linear a relationship between respiration rate and ammonia uptake in nitrogen-starved *Chlorella* cells. From his Fig. 5 (1953a), a ratio of 1.4 between NH_3 uptake and the extra oxygen consumption was calculated. This ratio is higher in maize roots than in *Chlorella* cells.

IV. *The effect of phosphate on the root respiration of phosphate-starved maize plants*

The effect of phosphate on the root respiration of phosphate-starved maize plants was studied in order to obtain additional evidence of a correlation between deficiency and respiratory response.

Phosphate deficiency could not be produced by transferring maize plants grown on a L.A. solution to L.A.-P solution, because these plants had a phosphate reserve due to surplus phosphate absorption in the preceding period. Nevertheless, phosphate deficiency was produced by raising maize seedlings in sand cultures which were periodically drained with L.A. solution with one of the following dilutions of its phosphate content: 0 P, 1/300 P, 1/100 P, 1/30 P and 1/10 P. The development of the plants treated with the four lowest phosphate concentrations was checked rather early. The plants treated with 1/10 P were only 50 cm high after about 10 weeks and also showed severe phosphate-deficiency symptoms. The roots of these plants were freed from sand by washing with L.A.-P solution, excised, and suspended in L.A.-P solution in the respiration flasks.

TABLE 15

Respiration rates of excised roots of phosphate-starved maize plants in response to the addition of phosphate. Roots were suspended in phosphate-free Long Ashton nutrient solution. Respiration rate was measured for 8 consecutive hours. Phosphate or L.A.-P (control) solution was added 3 hours after the start of the experiment. Concentrations are the final values after salt addition.

Addition	Respiration rate during 8 consecutive hours in cu.mm O_2/hr/mg dr.wt roots							
	1	2	3	4	5	6	7	8
L.A.–P (control)	1.42	1.50	1.54	1.58	1.60	1.65	1.68	1.70
%	*92*	*97*	*100*	*103*	*104*	*107*	*109*	*110*
0.005 M NaH_2PO_4 . .	0.76	0.89	0.91	1.25	1.35	1.44	1.39	1.42
0.01 M „ . . .	0.88	1.04	1.08	1.49	1.49	1.57	1.56	1.54
0.02 M „ . . .	1.43	1.51	1.54	2.05	1.99	2.04	2.03	2.16
Mean	1.02	1.15	1.18	1.60	1.61	1.68	1.66	1.71
%	*86*	*97*	*100*	*136*	*136*	*142*	*141*	*145*
% resp. increase	—	—	—	*33*	*32*	*35*	*32*	*35*

These phosphate-deficient roots showed a respiration rate of only 20–30 % of sand-cultured control plants. The addition of phosphate to the phosphate-deficient roots gave a respiratory increase of 32–35 % above the response to the addition of L.A.-P solution (Table 15). Of the salts tested (NaH_2PO_4, NaCl, Na_2SO_4, $NaNO_3$), only NaH_2PO_4 gave a respiratory response.

Chapter IV

DISCUSSION OF THE RESULTS

§ 1. Relation between rate of ammonium ion uptake and ammonium ion concentration; properties of the first binding

In this and the following paragraph, the results of Chapter II and III are discussed. Experiments with intact maize plants showed that the relation between the rate of NH_4^+–ion uptake and NH_4^+concentration gives a rectangular hyperbola as presented in Fig. 2 (p. 29). This curve shows some resemblance to an adsorption curve, *e.g.* an adsorption isotherm according to the equations of Freundlich or Langmuir. In order to check as to which of the two equations agrees better with the data obtained, a comparison was made with both isotherms.

According to Freundlich's adsorption isotherm, the relation between the rate of ion uptake (u) and the external ion concentration (c) can be expressed by the formula:

$$u = k.c^{1/n} \quad (cf. \text{ Kruyt, 1946}).$$

Whether or not the data obtained fit this equation can be readily seen by plotting them logarithmically. According to Freundlich's isotherm, a straight line ($\log u = \log k + 1/n \log c$) should be obtained. The data are represented logarithmically in Fig. 7.

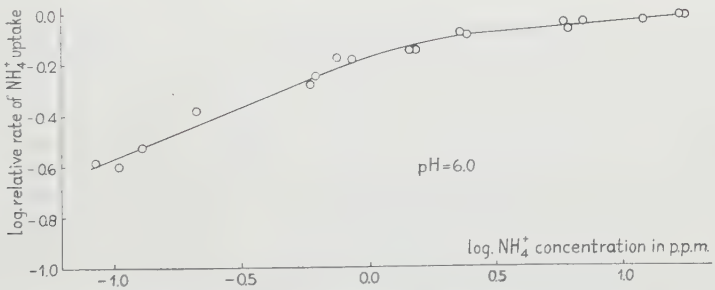


Fig. 7. Relation between log. ammonium ion concentration and log. relative rate of ammonium ion uptake, at pH = 6.0, 20° C.

From this graph, it appears that at sufficiently low NH_4^+concentrations the rate of NH_4^+–ion uptake follows Freundlich's curve (*i.e.* gives a straight line), but this is not the case at higher NH_4^+concentrations. At the higher NH_4^+concentrations the exponent $1/n$ of the Freundlich equation is no longer constant, but gradually decreases to zero. This suggests that at the higher NH_4^+ concentrations a saturation of the adsorbent (carrier system) is reached. Such a phenomenon is well-known in adsorption reactions.

An adsorption curve which shows a saturation effect at the higher concentrations is represented by Langmuir's isotherm. Moreover, Langmuir's adsorption equation has a theoretical foundation, whereas Freundlich's adsorption isotherm is only an empirical expression.

Langmuir's adsorption isotherm can be represented by the formula:

$$q = \frac{p.c}{c + k} \text{ (cf. KRUYT, 1946),}$$

where q = amount bound carrier, p = total amount carrier, c = ion concentration and k = a constant. In relation to ion uptake, the asymptotical course of this curve can be explained by assuming a binding of NH_4^+ions to a limited number of active sites or carriers in the protoplasm. This carrier hypothesis, and the likelihood for a first binding on the outer side of the protoplasm, are discussed in Chapter I.

Because the relative rate of ion uptake can be considered to be proportional to the amount of bound carrier (picture of the revolving belt), the Langmuir equation can also be applied to ion uptake. The revolving belt picture does not necessarily imply a spatial removal of ions from the outside surface, it might equally well represent a removal by a chemical (enzyme) reaction. In fact, Langmuir's equation is frequently applied to describe the velocity of enzyme reactions (*cf.* UMBREIT *et al.*, 1948) in which the Michaelis-Menten enzyme constant (k) is comparable to that ion concentration at which half the limiting absorption rate is found. This concentration we will call the "half value" concentration. Evidently, at this concentration the hypothetical carrier system is loaded with ions to half its maximal capacity.

The formula of the Langmuir equation shows that the relation between the reciprocal uptake ($:1/q$) and the reciprocal concentration ($1/c$) is linear. Therefore, if they agree with Langmuir's equation, the reciprocal values of the data should fit a straight line. This was checked by determining the line of best fit, which is shown in Fig. 8.

In the calculation of the linear regression line, the values obtained under standard conditions (Chapter II, § 6) were not incorporated, because they had been used for the calculation of the relative uptake values. This treatment was satisfactory since it is chiefly the points at low ion concentration, *i.e.* in the bend of the hyperbolic curve, which determine the slope of the regression line. It appeared that the NH_4^+–ion uptake values near the saturation level deviated from this regression line by a statistically significant amount, whereas at the lower NH_4^+ concentrations the distribution of the points along the line was normal and thus agreed with a linear regression.

As illustrated in Fig. 8, there is a distinct bend in the line at the higher concentrations. Thus it can be concluded that the uptake-concentration curve for NH_4^+ lies between a Freundlich and a Langmuir equation.

Freundlich's isotherm does not fit the data well, because at the higher NH_4^+ concentrations the uptake was lower. On the other hand, Langmuir's isotherm does not fit completely either, because at the higher concentrations there was no absolute saturation effect, since there was still a small increase in NH_4^+–ion uptake at increased NH_4^+ concentrations. An explanation of the latter phenomenon can be

derived from VAN DEN HONERT's (1954) work. He observed that salt uptake in potato storage tissue did not show a saturation effect at higher ion concentrations, but the uptake continually increased till a very high concentration was reached. This "potato effect" was explained as an accumulation of salt by progressively deeper cell layers. A slight potato effect may have caused the increased NH_4^+–ion uptake at higher NH_4^+concentrations observed in maize roots. If we assume that the deviation from Langmuir's curve (*i.e.* no saturation value)

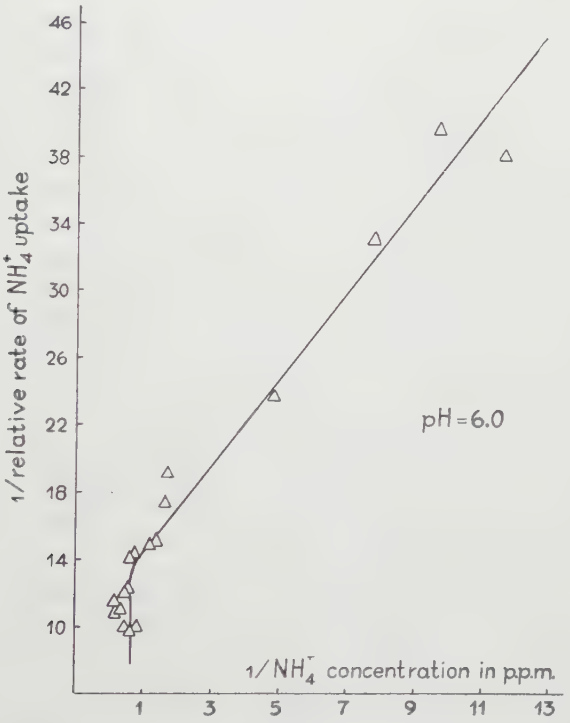


Fig. 8. Relation between reciprocal values of ammonium ion concentration and relative rate of ammonium ion uptake, at pH = 6.0, 20° C.

at the higher ion concentrations is due to participation of deeper cell layers in the ion uptake process, then it may be concluded that if it was possible to measure the ion uptake at the root surface, the ion uptake values would correspond better to Langmuir's equation than to Freundlich's equation.

Such a Langmuir isotherm could be characterized by its "half value" concentration. This half value concentration, as computed from the linear regression line, is 0.23 $\pm$ 0.02 p.p.m. NH_4^+ at a pH = 6.0 and root temperature of 20° C. The half value concentration would then correspond to the NH_4^+ concentration at which the revolving belt is loaded to half its loading capacity. Another charac-

teristic feature is the saturation value, which is almost reached at the rather low NH_4^+ concentration of 10 p.p.m. The half value virtually determines the shape of the ion uptake-concentration curve, and thus gives some information about the nature of the ion uptake process and the properties of the assumed carriers. It may be emphasized that this and similar information has been obtained indirectly by merely changing the environmental conditions of the roots of *intact* maize plants.

Van den Honert *et al.* (1955, unpublished), working with maize in static water cultures and short experimental periods, found a half value concentration which was about 6 times as high (*i.e.* 1.4 p.p.m. NH_4^+) as the value found in the present study with flowing-water cultures. With the constant-flow water culture technique used in this study, a new steady state took as much as 24 hours to become established. It may therefore be possible that long-period experiments with the flowing-water culture technique allow a better unloading of the "revolving belt" inside the cell, whereas in short-time experiments the revolving belt will return partly loaded to the outside. This would imply a lower rate of ion uptake, especially at the lower ion concentrations. The latter phenomenon might also be expressed as an after-effect of the preceding "high-salt" condition of the root tissue involved in the subsequent measurement of the rate of ion uptake (*cf.* Hoagland *et al.*, 1944). Helder (1952, Fig. 5) showed a similar change in half value concentration for the phosphate uptake by maize plants in connexion with the external supply of sugar to the roots. Epstein and Hagen (1952), applying the reciprocal values of Langmuir's equation, observed for the rubidium ion uptake by excised barley roots quite different half value concentrations. They attributed these deviations in the various experiments to differences in hereditary factors and pretreatment of the barley strains used.

Van den Honert *et al.* (1954) observed that the NH_4^+–ion uptake by maize roots is to a great extent unaffected by the presence and uptake of other ion species. This suggests a very specific binding between carrier and NH_4^+ions.

As shown in Fig. 3 (p. 30), the NH_4^+–ion uptake-concentration curve obtained at pH = 4.6 is equal to that found at pH = 6.0.

This suggests that the carrier-ammonium ion binding is not influenced by a hydrogen ion concentration of $10^{-4.6}$ M. At a pH = 4.0, a much lower NH_4^+–ion uptake was observed. However, the significance of this result is very doubtful in view of the injurious effect of this low pH on the root. Evidence pointing in the same direction is the inhibition of root respiration at low pH values (Chapter III, III).

Van den Honert *et al.* (1955, unpublished) found that at high pH values there is not only an active ammonium ion uptake but also a passive molecular ammonia diffusion, proportional to the concentration of free ammonia available. Experiments at higher pH values were not performed, since passive ammonia uptake was not the subject of this investigation.

§ 2. The hydrogen ion release in relation to ammonium ion uptake

In Fig. 2 (p. 29), the quantitative relation between hydrogen ion release and ammonium ion uptake is given. This Figure shows, at concentrations below 3 p.p.m. NH_4^+, a NH_4^+–H^+ exchange in the ratio of practically 1 : 1, whereas at NH_4^+ concentrations above 3 p.p.m. the H^+–ion release is about 80 % of the NH_4^+–ion uptake. At still higher NH_4^+concentrations, up to 20 p.p.m., this ratio tends to decrease further to about 70 %.

As already stated in Chapter II, the base-excess determination for H^+–ion release actually measures the net effect between cation and anion uptake. A H^+–ion release equivalent to the NH_4^+-ion uptake, as observed in the present study at low NH_4^+ concentrations, clearly suggests that the NH_4^+–ion uptake is independent of the anion uptake. This is in contradiction to Lundegårdh's "anion respiration" hypothesis (see Chapter V). An exchange of 80 % H^+ ions for NH_4^+ ions indicates that an appreciable anion uptake is involved, *i.e.* an anion uptake equivalent to 20 % of the NH_4^+–ion uptake. In the present experiments increased NH_4^+–ion concentrations were obtained by introducing more ammonium salt to the nutrient solution. Therefore, a decrease in the ratio of the NH_4^+–H^+ exchange at the higher NH_4^+–ion concentrations can be explained by an ever-increasing anion uptake, while the NH_4^+–ion uptake has already reached its saturation value.

In the NH_4^+–ion uptake-concentration curve at pH = 4.6, a few H^+–ion release figures are plotted. As far as can be judged from these six points, no difference can be observed in the H^+–ion release between pH = 4.6 and pH = 6.0 (compare Fig. 2 and Fig. 3). This evidence seems acceptable, because the NH_4^+-ion uptake is also unaffected by a pH = 4.6. Even at pH = 4.0, where there is a profound inhibition of NH_4^+–ion uptake due to root damage, the ratio between NH_4^+–ion uptake and H^+–ion release was not found to be changed (data not given).

In Table 4 (p. 33), the ratio between H^+–ion release and NH_4^+–ion uptake is given for one batch of maize plants at different ages. These figures demonstrate that in vigorously growing maize plants (early September) this ratio is about 85 % at the higher NH_4^+concentrations. This ratio is consistent with that formerly obtained (see Fig. 2). However, a month later (early October), the ratio decreased to 75 % and three weeks later (late October) a ratio of only 32 % was reached. Hence, in addition to the evidence that in older plants the NH_4^+–ion uptake decreases (Chapter III), there is also a considerable change in the ratio between H^+–ion release and NH_4^+–ion uptake.

This evidence can be explained by a less selective ion uptake, *i.e.* the entrance of NH_4^+ ions together with anions. This suggestion is consistent with results of Broyer and Hoagland (1943), where—in contrast to young barley roots—a more passive permeability for both ions of salts was observed in older barley roots.

Nevertheless, a closer examination of the H^+–ion decrease in the

present experiments with intact maize roots showed that the anion uptake was *not* appreciably increased. This effect was found to be due to a distinct K^+–ion release by the roots, even as much as 1–2 p.p.m. K^+/hour per plant. Thus in older maize roots there was a considerable exchange of NH_4^+ ions for K^+ ions, while in vigorously growing plants K^+ release was negligible. Luttkus and Böttticher (1939), Humphries (1950, 1951, 1952), etc. also state that potassium is easily released by roots. In these experiments with maize roots a K^+–ion release occuring simultaneously with a Na^+–ion uptake was noted. This is in agreement with observations of Scott and Hayward (1954) in *Ulva lactuca* and Cowie *et al.* (1949) in *Escherichia coli*, where separate mechanisms regulating potassium ion and sodium ion uptake were suggested. A phenomenon like the NH_4^+–K^+ exchange in older maize roots was observed by Jacobson and Ordin (1954) in the older roots of Romaine lettuce. According to these investigators, as much as 84 % of the K^+–ions taken up from a $KHCO_3$ solution by these roots was exchanged for Ca^{++}ions and Mg^{++} ions. However, in barley seedling roots, during K^+–ion uptake, a K^+–H^+ exchange was predominantly observed.

It must be emphasized that one should be careful not to confuse the exchange reactions at root surfaces, which can be measured as static equilibria in experiments such as those of Williams and Coleman (1950), with an ion-binding to a carrier system. Most probably the adsorption found in these experiments had nothing to do with a carrier system, because acid groups of pectin substances in the cell walls may have been involved. Nor does it follow from a H^+–ion release equivalent to NH_4^+–ion uptake, that this phenomenon is merely based on an ion exchange reaction comparable to that in ion exchange resins as suggested, for instance, by Jacobson *et al.* (1950). It may be equally possible that an enzymatic binding reaction is the first step in NH_4^+-ion absorption, as discussed in Chapter II. This view is not so extraordinary, since enzymes have been shown to be active in cell surfaces (*cf.* Rothstein and Meier (1948), and Street and Lowe (1950).

Also, the H^+–ion release could be much more intimately linked to metabolism than would be evident at first sight. Metabolically active plants showed a far greater H^+–ion release than older plants. Moreover, as the protoplasm has only a small buffer capacity towards the alkaline side (*cf.* Burström, 1945), a continuous large-scale release of H^+ ions must be counterbalanced by a special metabolic production of H^+ ions. Therefore, in the opinion of the present author, not only is the NH_4^+–ion uptake a metabolically-linked active process, but the H^+ ions also seem to be metabolically produced.

§ 3. The effect of salts—especially ammonium salts—on root respiration; the evidence of a "cation-induced respiration"

As is well-known, Hoagland's "low-salt, high-sugar" roots had a much greater capacity for ion absorption than his "high-salt, low-sugar" roots. Therefore, a certain degree of salt deficiency seems to be essential for a high salt uptake.

The same phenomenon holds for storage tissue where such a deficiency may be obtained by prolonged washing of the tissue disks with distilled water or running tap water, as shown by STEWARD *et al.* (1943) and SUTCLIFFE (1952, 1954 *a*, *b*). Steward *et al.* observed that a longer washing time produced a greater intake of bromide in a given time. This enhanced absorption due to prolonged washing of the tissue has been demonstrated by other workers, *e.g.* ASPREY (1937), STILES and DENT (1946), and REES (1949). In the same way, SUTCLIFFE (1952) demonstrated that the initial rate of potassium ion uptake in red beet root storage tissue was a function of its salt and solute deficit created by protracted washing.

In "salt respiration" experiments, LUNDEGÅRDH *et al.* (1933) gave their wheat roots a pretreatment of 24 hours in distilled water, and ROBERTSON *et al.* (1948*b*) their carrot storage tissue a washing with running tap water of as much as 120–350 hours. The treatment with distilled water is an indispensable prerequisite for a measurable respiratory response. The pretreatment used by Lundegårdh and Robertson in their respiration experiments very probably created a "low-salt" condition in the tissue by either the removal of salt to the shoot or leakage from the tissue to the medium. It might well be that a "low-salt" condition in these tissues is not only a requisite for rapid salt uptake but also for respiratory response. Indeed, SUTCLIFFE (1952) demonstrated that the salt-induced cyanide-sensitive component of respiration was the more conspicuous the longer the tissues were pretreated with distilled water.

HOAGLAND *et al.* (1936, 1939, 1940, 1944) and STEWARD *et al.* (1936, 1937, 1940, 1943) have given evidence that a "low-salt" condition is connected with a low metabolic status of the tissue involved. Therefore, it seems reasonable to expect a similar connexion between metabolic status and "salt respiration". The question arises as to whether salt deficiency is a requisite for "salt respiration". In this case a respiratory response could be expected only after a supplement of the deficient ion, whereas other ion species would have no influence on the respiration rate. This question is treated in the experiments described in Chapter III.

The addition of ammonium salts to maize roots in "high-salt, high-sugar" condition, *i.e.* maize radicles, did not result in an increase of the rate of root respiration (Table 5), probably because of the high nitrogen content of these roots. However, roots of 16-week old maize seedlings, previously grown in a Long Ashton nutrient solution, show a pronounced increase (12–27 %) in root respiration rate to a supplement of ammonium salts after a starvation period of 2–7 days in a very dilute calcium sulfate solution (Table 6). Distilled-water pretreatment was not used in these experiments because it proved to be injurious to the roots. A similar increase of root respiration due to ammonium salt addition is also produced by a pretreatment in a Long Ashton minus N nutrient solution (Table 7). In the experiment presented in Table 8 it was observed that $(NH_4)_2SO_4$ and not an equimolar quantity K_2SO_4 gave a respiratory increase. Evidently, anion (*i.e.* SO_4^{--}) uptake

was not responsible for the increased root respiration rate, but NH_4^+-ion uptake alone (Table 8). As will be discussed later (Chapter V and VI) this result is not consistent with Lundegårdh's "anion respiration" hypothesis.

In the preceding experiments, the root respiration was measured in distilled water prior to the addition of salt. In the next experiments, the nitrogen-deficient roots were suspended in the respiration flasks in a Long Ashton minus N nutrient solution. In this way, a specific respiratory response to ammonium salt addition was demonstrated, because many other nutrient salts were already available to the roots (Table 9). Moreover, the general effect of nitrogen salts in the case of nitrogen-starvation was observed, since nitrate had a similar effect on the respiration rate (Table 9). In contrast to the supply of $(NH_4)_2SO_4$ or $NaNO_3$, *no* respiratory effect was produced by the addition of either Na_2SO_4 or K_2SO_4. A very small respiratory increase in response to the addition of NaCl was observed in these nitrogen-starved roots (Table 9).

It is noteworthy that the respiratory increase due to nitrate addition rose much more slowly than the increase produced by ammonium salt addition. This is probably due in part to the relatively slower rate of nitrate uptake compared to ammonium ion uptake, and in part to different pathways of nitrate and ammonium nitrogen in metabolism. However, the increased respiration induced by nitrate was maintained much longer than that in response to ammonium salt addition. A possible explanation is that maize roots are better adapted to nitrate nutrition than to ammonium nutrition.

Experiments with roots exclusively grown in a Long Ashton nutrient solution were also performed for comparison with the experiments with nitrogen-starved maize roots. These roots were suspended in a Long Ashton nutrient solution. The addition of $(NH_4)_2SO_4$ showed a very small (2–5 %) respiratory increase, whereas the addition of $NaNO_3$ and NaCl did not affect the respiratory rate. However, the addition of K_2SO_4 gave a decrease of root respiration rate (Table 9).

The addition of sugars, with or without the simultaneous addition of ammonium salts and nitrates, was studied in normal roots and nitrogen-starved roots. In the normal roots there was a slight increase of the respiration after the addition of ammonium salts (Table 10). In these roots a considerable increase (50–65 %) of the root respiration rate was observed after the addition of sugar. The rise of respiration rate due to the addition of sugar by itself reached a level equal to that of sugar combined with ammonium salt (Table 10). This evidence indicates that the normal (Long Ashton) roots required sugar rather than ammonium salts for increasing their respiration rate.

In nitrogen-starved maize roots suspended in nitrogen-free Long Ashton solution, a respiratory increase of 20 % in response to ammonium salt addition was observed (Table 10). In contrast to the effect on normal roots, the addition of sugar alone did *not* give a large increase in the respiratory rate: after ten hours a respiratory increase of only about 40 % was observed. The addition of sugar combined

with either ammonium salt or nitrate, however, increased the respiration rate of these roots 110 % and 140 % respectively (Table 11). Here again, as in the case of the addition of nitrate alone, the addition of sugar combined with nitrate gave a slower increase in respiration rate than the corresponding addition of sugar combined with ammonium salt. However, the respiration increase due to the addition of sugar combined with nitrate reached a higher final level than that of sugar combined with ammonium salt, *i.e.* with nitrate 140 % against 110 % with ammonium salt (Table 11). This suggests once more a better utilization of nitrate nitrogen than ammonium nitrogen by maize roots. Moreover, the change in respiratory quotient (CO_2/O_2) observed indicates that nitrate as well as ammonium ions were involved in metabolism. In the nitrate experiment, the R.Q. increased from 0.88 to 1.23–1.25, whereas with ammonium salt addition the R.Q. decreased from 1.01–1.05 to 0.92–0.97 (Table 11).

Finally, experiments with synthetic ion exchange resins (Amberlites) were performed to demonstrate an ammonium ion uptake and respiration increase independent of the uptake of other ions. NH_4^+–bearing resins were obtained by percolating the H^+form of these resins in a column with NH_4OH solution. The NH_4^+–containing resins, suspended in distilled water, can be regarded as a *one-ion* NH_4^+solution, because the roots cannot absorb the large anions (particles) of the resin. The use of resins for obtaining a one-ion solution was adapted from the work of Jenny and Cowan (1933) with calcium-bearing clay particles in suspension, as mentioned in Chapter II, § 2. Moreover, similar experiments with synthetic ion exchange resins (Dowex 50 and XE–97) had already been performed by Epstein (1954) for potassium and calcium ion uptake in barley roots. In the present experiments, the resins Amberlite IR–120 and IRC–50 were used.

The root respiration of nitrogen-starved maize roots showed an increase of 25 % and 30 %, respectively, in response to the addition of these NH_4^+–bearing resins (Fig. 6, Tables 12 and 13). This respiratory increase was not influenced by the NH_4^+ concentrations (50–500 m.e./L) used, as can be seen in Tables 12 and 13. The smaller respiratory increase with Amberlite IR–120 is probably due to a lower exchangeability of ammonium ions on this strong-acid ion exchanger compared to the weak-acid ion exchanger Amberlite IRC–50. The addition of Na^+–bearing resin to nitrogen-deficient roots gave no respiratory response, whereas the addition of the H^+ form of these resins inhibited the respiration rate markedly, probably due to the injurious effect of these low pH's on the root (Tables 12 and 13).

Besides these experiments with nitrogen-starved roots, other experiments were performed with potassium- and phosphate-deficient roots in order to get a more general picture of the correlation between deficiency and "salt respiration".

Potassium starvation produced a significant respiratory increase of about 21 % compared to that of normal plants (Table 8). This is in striking contrast to nitrogen deficiency, where a respiratory decrease

of about 45 % was observed (Table 8). The addition of potassium salts decreased the enhanced respiration rate due to potassium starvation by 10 %. Therefore, the present results do not agree with observations of Steward *et al.* (1940, 1941, 1954) with potato storage tissue disks and those of Epstein (1954) with barley roots, which showed an increased respiration rate in response to potassium ion uptake. No explanation of this discrepancy can be suggested. However, the present observation is supported by the work of Gregory and Richards (1929), Richards (1932), and Gregory and Sen (1937), which showed that potassium deficient barley leaves had an increased respiration compared to the respiration of normally dressed plants. Mulder (1955) found an increased respiration rate in tubers and storage tissue slices of potassium-deficient potato plants compared to that of fully potassium-fertilized potato plants grown on the same field. He concluded, however, that this effect was only due to the fact that potassium-deficient and normal potato tissues differ in their sensitivity to bruising. Pirson and Seidel (1950) observed a higher respiration rate in potassium-starved *Lemna minor* roots than in normal ones. The effect is also known in some algae. Pirson, Tichy and Wilhelmi (1952), and Neeb (1952) observed that potassium starvation induced a greater respiration rate in *Ankistrodesmus* and *Hydrodictyon*.

Phosphate deficiency and its influence on root respiration in maize was also investigated. Phosphate-free pretreatments of maize plants previously grown in a Long Ashton nutrient solution did not produce phosphate deficiency. This may be explained by the assumption of an initial surplus phosphate uptake (*cf.* van den Honert, 1933, for sugar cane). Phosphate-deficient maize plants were obtained by cultivation in sand cultures drained with low-phosphate or phosphate-free solutions. The excised phosphate-deficient roots, suspended in a Long Ashton minus P nutrient solution in the respiration flasks, showed a respiration rate of only 20–30 % of that of Long Ashton roots. Only phosphate salt addition produced a respiratory increase of 35 % above control. Other salts did not affect the respiration rate of phosphate-starved maize roots. A reduced respiration due to phosphate-starvation is also reported in oat leaves by Petrie and Williams (1938). Here too, treatment with phosphate increased considerably the low initial respiration rate.

§ 4. Additional discussion of the literature on nitrogen-starvation and respiration

For the sake of clarity, a number of citations of literature were omitted in the preceding section and they will be briefly summarized here. Most of these investigations had results consistent with those of the present study reported in the previous section.

Gregory and Richards (1929), Richards (1932), and Gregory and Sen (1937) demonstrated a marked reduction of the respiration rate in nitrogen-starved barley leaves. Petrie and Williams (1938) observed a low respiration rate in nitrogen-deficient Sudan grass

leaves if respiration was computed per unit of dry matter. However, calculated on a protein basis, the nitrogen-deficient tissue showed considerably higher respiration values than normal tissue. Apparently this is due to the fact that in non-deficient plants a part of the protein was inactive in metabolism. Subsequent nitrogen treatment caused a marked respiration increase.

MULDER (1955) observed a small reduction in respiration rate in some experiments with tubers and storage tissue slices of nitrogen-deficient potato plants. In some of his other experiments, however, tuber tissue from healthy and deficient plants showed no difference in respiration rate, although these potato plants had pronounced nitrogen-deficiency symptoms.

HAMNER (1936) found a low respiration rate in nitrogen-deficient wheat and tomato roots. The roots of these minus-nitrate plants showed a considerable increase in carbohydrate content, but were low in nitrate and soluble nitrogen content. Thus, the presence of carbohydrates alone does not necessarily imply an increased respiration rate. The addition of nitrates to the roots of these plants caused an increased root respiration. The greater the amount of reserve carbohydrates available, the more immediate the response and the greater in degree.

WHITE (1936) and WHITE and TEMPLEMAN (1937) reported for nitrogen-deficient *Lemna minor* plants an increased sugar content of the tissue due to the fact that nitrogen-starvation markedly affected the rate of development of new fronds, but did not reduce the rate of photosynthesis. The rate of respiration in the fronds was appreciably reduced. Subsequent addition of nitrogen salts increased the respiratory rate and decreased the sugar content of the tissue. It was noteworthy that the depression of respiration during nitrogen-starvation was only of the order of 25 %, whereas the rise in respiration rate, following transfer of a starved colony to a solution with full nutrient supply, was of the order of 300 %.

HOAGLAND *et al.* (1939, p. 1031; 1944, p. 142) observed that in excised barley roots the addition of nitrate and ammonium nitrogen produced a marked increase in respiration rate over that in distilled water. In some of his experiments ammonium nitrogen seemed to stimulate the rate of respiration to a greater extent than nitrate, although the latter also had an appreciable effect. WILLIS (1951), FOLKES, WILLIS and YEMM (1952), and WILLIS and YEMM (1955) consistently observed that in nitrogen-starved barley seedling roots the application of ammonium or nitrate salts produced a considerable stimulation of the respiration rate. From the graphs presented by WILLIS and YEMM (1955) it appeared, moreover, that the respiratory increase due to ammonium salt addition was faster than the increase due to nitrate addition. However, the respiration rate finally obtained in the case of ammonium nitrogen addition reached a lower level than that in response to nitrate addition. These observations are in agreement with the present results obtained with nitrogen-deficient maize roots, presented in Chapter III.

SYRETT (1953 *a*, *b*) observed in *Chlorella pyrenoidosa* that the addition

of ammonium sulfate increased the respiration rate markedly in nitrogen-starved cells. Glucose addition increased the respiration rate in these cells to some extent, but the respiration rate was further increased by combined glucose and ammonium nitrogen addition. The respiration rate then reached was much the same as when ammonium salt alone was added. The increased respiration rate continued only as long as ammonium nitrogen was being assimilated. A linear relationship between ammonium nitrogen assimilation and the rate of oxygen consumption was found (Syrett, 1953 *a*, Fig. 5). This ratio was much lower than that observed in the present study with nitrogen-starved maize roots (Chapter III, Table 14).

Yemm and Folkes (1954) reported that nitrogen-deficient food yeast, *Torulopsis utilis*, suspended under aerobic conditions in a carbohydrate-free medium, responded rapidly to the addition of ammonium phosphate. Here too, the increased respiration rate was accompanied by ammonium nitrogen assimilation, and a close quantitative relationship between these two processes was demonstrated. Respiratory increases induced by ammonium ion uptake were also observed in some bacteria, *e.g. Serratia marcescens* (McLean and Fisher, 1947, 1949), *Escherichia coli* (Armstrong and Fisher, 1947) and some *Rhizobium* species (Burris and Wilson, 1952).

Recently Austin (1955) demonstrated a respiratory stimulation in response to the addition of ammonium salt or nitrate combined with sugar in excised roots of barley seedlings. Ten hours subsequent to substrate addition, a respiratory increase of 40–45 % with ammonium sulfate and 60–65 % with potassium nitrate was observed. These values are rather low as compared with those of the present study in similar conditions and experimental time. In the present study with excised maize roots, a respiratory increase of 110 % and 140 % was obtained with ammonium salt and nitrate addition, respectively (Table 11). This suggests the possibility that the barley roots used by Austin (1955) were insufficiently nitrogen-starved. Austin also observed an increase or a decrease of the respiratory quotient (R.Q. = CO_2/O_2) in response to the supply of nitrate or ammonium nitrogen, respectively. Warburg and Negelein (1920), Cramer and Myers (1948), and Davis (1953) consistently found in *Chlorella* a R.Q. increase due to nitrate absorption. Gilbert and Shive (1945) found a similar R.Q. increase due to nitrate uptake by soya bean, oat, and tomato plants in water culture. A R.Q. decrease with ammonium nitrogen uptake and assimilation was demonstrated by Syrett (1953 *a*, *b*) in *Chlorella*, and by Yemm and Folkes (1954) in *Torulopsis*. The results of the present study (Table 11) are in agreement with the observations cited above, where a reverse shift of the respiratory quotient due to nitrate or ammonium nitrogen uptake was found.

Warburg and Negelein (1920), Gilbert and Shive (1945), and Bonner (1950) suggested that the extra output of carbon dioxide during nitrate uptake must be due to the fact that a part of the oxygen of the nitrate serves as a hydrogen accepter in respiration and substitutes for a portion of the free oxygen which would otherwise be

consumed. However, another view may be equally valid. Ulrich (1941) observed that the respiratory quotient of young barley roots was influenced by selective ion uptake. When cations were absorbed in excess of anions, organic acids were formed to counteract an increase in pH of the root sap. Conversely, when anions were absorbed in excess of cations, organic acid anions tended to disappear, leaving the bases to balance the increase in inorganic anions. These shifts in organic acid content were reflected in the R.Q. values. The R.Q. value was less than one when organic acids were formed and greater than one when the organic acid content decreased. Therefore, a greater carbon dioxide production during nitrate uptake can be also associated with organic acid breakdown. The relation of organic acid metabolism to selective ion uptake is confirmed by the work of H.E. Clark, (1936), Vickery *et al.* (1940), Machlis (1944), Burström (1945), and Jacobson and Ordin (1954).

§ 5. Conclusions

From the experiments, apart from any existing theory on ion uptake, the following conclusions can be drawn:

The experiments on the ammonium ion uptake in intact maize plants (Chapter II) suggest an *active* ammonium ion uptake *independent* of the uptake of other species of ions. Moreover, the results with excised roots (Chapter III) favour the concept of a "*cation-induced respiration*", *i.e.* an increased respiration rate due only to an active ammonium ion uptake. In addition, evidence was also obtained that in the case of nitrogen salt or phosphate uptake, a respiratory increase is only obtained when the tissue is deficient in these particular elements. Therefore, we have not only a specific ion uptake (Chapter II) but also a specific respiratory increase in response to the uptake of the ion in question. The present results tend to link the respiratory response to deficiency-influenced metabolism rather than to salt transport.

These conclusions, *i.e.* active ammonium ion uptake, cation respiration, and the connexion between salt respiration and salt deficiency in metabolism, are evidently inconsistent with Lundegårdh's "anion respiration" theory and more in agreement with Steward's views.

Because so much research work has been based on Lundegårdh's important hypothesis, a closer examination of this hypothesis may be justified. This will be done in the next chapter.

Chapter V

LUNDEGÅRDH'S "ANION RESPIRATION" HYPOTHESIS

§ 1. A critical survey

Fundamentally, Lundegårdh's (1933) hypothesis deals with anion uptake and transport coupled to electron transfer in the cytochrome-cytochrome oxidase system. This hypothesis in its original form emphasizes that only anion uptake is active, while the cations are passively moved along by the electrical gradient created by the anion transport.

Consequently, such a mechanism will only accumulate anions and cations in equimolar quantities. Therefore, LUNDEGÅRDH (1949*b*, 1950*b*) can explain an excess cation uptake only by the assumption that "native anions", *i.e.* organic acid anions, are produced in the cytoplasm in amounts equivalent to the excess cation absorption. Moreover, the transport of anions coupled to electron transfer should in principle be non-specific, so anions of the same charge would be transported at equal rates. A summary of the evidence pro and contra this hypothesis may be helpful.

Supporting Lundegårdh's hypothesis are the following observations. Anion and cation uptake and transport are separate processes; evidence for this view seems well established. The surface membrane of living roots has a negative charge which will hamper anion uptake because of the repelling force of two similar charges. Therefore, energy has to be expended to overcome this force, while cation uptake is facilitated by the attraction of opposite charges. Accordingly, WANNER (1948) found high "metabolic" temperature coefficients for anion uptake and low non-chemical ones for cation uptake.

The observation that salt uptake is correlated with increased respiration seems to be well established (*cf.* LUNDEGÅRDH *et al.*, 1933 etc.; ROBERTSON *et al.*, 1940 etc.; HOAGLAND *et al.*, 1944; STEWARD *et al.*, 1932 etc.; VAN EIJK, 1939; SUTCLIFFE, 1952, 1954 *a*, *b*; LEWIS, 1955; and the present study). However, LUNDEGÅRDH (1933) made the distinct restriction that only anion uptake shows a relation to respiration; whereas according to him, cation uptake does *not*. This is the basis of Lundegårdh's "anion respiration" theory of anion transport in relation to a cytochrome-cytochrome oxidase system. This point of view is strongly supported by the evidence that salt accumulation is inhibited by cyanide, while a cyanide non-sensitive fraction of the respiration persists. From this, it can be concluded that only the cyanide-sensitive fraction of the aerobic respiration is related to ion uptake. This observation was confirmed by the work of ROBERTSON *et al.* (1945*a*, 1948*b*) and SUTCLIFFE (1952, 1954*a*, *b*). Other specific inhibitors of cytochrome oxydase-iron, *e.g.* CO (light reversible), NaN_3, NaF and α, α'-dipyridyl, have the same effect, as shown by LUNDEGÅRDH (1949*b*, 1954), ROBERTSON *et al.* (1948*a*), and STENLID (1950).

Moreover, ROBERTSON *et al.* (1948*b*) called attention to the fact that 4 electrons are required for the reduction of 1 molecule of oxygen, so that theoretically 4 anions will be transported for 1 molecule of oxygen consumed. Actually, Robertson, working with slices of carrot storage tissue, observed that the high value of 4 was approximated only at higher salt concentrations. This observation is thus consistent with Lundegårdh's hypothesis.

Against Lundegårdh's view, the following arguments may be cited. Although a correlation between ion uptake and respiration seems to be well established, Lundegårdh's statement that only the absorption of anions shows a relation to respiration is not justified, as even his own figures show. From his graphs LUNDEGÅRDH (1940, Fig. 19 and 20) concluded that anion uptake shows a linear relation to respiration

and cation uptake does *not*. However, these graphs, reproduced here in Figures 9 and 10, show that he separated the anions according to their kind, but did not follow the same treatment for the cations.

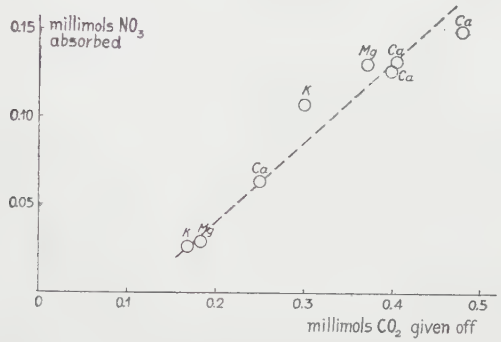


Fig. 9. The relation between anion absorbed and CO_2 eliminated, after LUNDEGÅRDH (1940, Fig. 19).

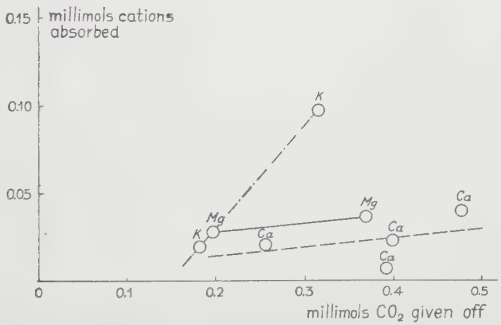


Fig. 10. The corresponding relation between cations absorbed and CO_2 eliminated, after LUNDEGÅRDH (1940, Fig. 20). The regression lines are drawn by the present author.

Therefore, it is reasonable that a more scattered picture is obtained for the cation relation than for the anion relation. If we compile all the data for different kinds of anions in one graph, we will obtain an analogously scattered picture, while if we separate the cations according to their species, as in Fig. 10, we get a picture similar to that obtained for the anions. Thus, Lundegårdh's figures give no evidence of an essential difference between anion and cation uptake in relation to respiration. It may be recalled that for some animal cells it has been proposed that there is an active movement of cations rather than anions (*e.g.* SOLOMON, 1952).

Moreover, following Lundegårdh's hypothesis, the k-values in his formula : $R_t = R_g + k.A$ (R_t = total respiration, R_g = ground respiration, A = anion uptake) will be the same for anions of the same charge, because only charge is the driving force in anion transfer. But Lundegårdh himself found different k-values for NO^-_3 and Cl^-

uptake, as demonstrated by the different slopes (slope $= 1/k$) of his linear regression lines for these ions (Lundegårdh, 1933, Fig. 3, pp. 242, 249–251; 1940, Fig. 19–21, pp. 316–317 and 1949, p. 383). However, the different k-values indicate a more specific anion uptake, a feature for which Lundegårdh's hypothesis leaves no room.

An acceptable explanation of the different slopes of the "anion uptake-respiration" regression lines, however, can be found. Lundegårdh gave for the *k*-values (*i.e.* rate of respiration/rate of ion uptake) in his formula for NO_3^-, Cl^-, and $SO_4^=$ the ratio of 2 : 3 : 6. Accordingly, the slopes of the regression lines (*i.e.* $1/k$ = rate of ion uptake/rate of respiration) were in the proportion of 1/2 : 1/3 : 1/6, *i.e.* of 3 : 2 : 1. Lundegårdh added that in the same material the relative absorption rates of NO_3^-, Cl^-, and $SO_4^=$ were in the ratio of 3.7 : 2.1 : 1. Therefore, the ratio of the quotient ion uptake/respiration and the ratio of relative absorption rates were approximately the same. Thus, the rate of respiration must have been about the same in each case, a suggestion strongly supported by the work of Robertson *et al.* (1948*b*) and Sutcliffe (1952) which will be discussed later. The divergent slopes of the regression lines seem, therefore, mainly caused by the differential absorption rates of the ions involved.

A similar argument would explain the divergent slopes of the cation uptake-respiration regression lines computed from Lundegardh's data (see Fig. 10). Here, the *k*-values for K^+, Mg^{++} and Ca^{++} were in the ratio of 2 : 20 : 21. Hence, the $1/k$-values (*i.e.* ion uptake/respiration) were in the proportion of 1/2 : 1/20 : 1/21. These latter figures were consistent with the ratio of relative absorption rates of these cations, *e.g.* K^+ions are accumulated many times faster than Mg^{++} or Ca^{++}ions.

The close correlation between activity of cytochrome and salt uptake which has been demonstrated in great detail by Lundegårdh (1945; 1950*a*; 1952; 1953*a*, *b*; 1954) cannot be regarded as a proof of the theory of "anion respiration", *i.e.* an anion *transport* achieved by the cytochrome system. Such results can equally well be interpreted as indicating that salt accumulation is in some way linked to the energy-yielding respiration process. Moreover, cytochrome is involved in the enzymatic processes of nitrate reduction (*cf.* Taniguchi *et al.*, 1953; Erkama *et al.*, 1954 and Verhoeven *et al.*, 1956) and it has even been suggested that "cytochrome *b*" is identical with nitrate reductase (*cf.* Sato *et al.*, 1949; 1952).

Further, the structural position of the cytochrome system in the cell can be considered in two ways. First, the cytochrome-cytochrome oxidase system may be structurally oriented in the outside membrane with a cytochrome oxidase facing the medium and a cytochrome *b* facing the place of accumulation. Second, it may be more diffusely scattered in the cell, *e.g.* situated in the mitochondria (*cf.* Burström, 1954, p. 298, discussing Lundegårdh's view), and the anion uptake would proceed along an oxidation gradient. In the latter case the anions would enter the cell, where the highest O_2 tension prevails, and be transported to the inside, where a lower O_2 tension exists, as

visualized in Lundegårdh's diagrams (1940, Fig. 34, p. 370; 1945, p. 26 and 1950, Fig. 2, p. 109).

In contrast to this conception, in some plants adapted to flooded soils, *e.g. Cladium mariscus* (V. M. Conway, 1936, 1937) and rice plants (van Raalte, 1940), the oxygen is transported from the aerial parts of the plant through large air channels in the tissues to the roots. Here, the epidermal cells of the root derive their oxygen supply from the inner side of the epidermal layer, while the uptake of ions continues from the outside. With this situation, ion uptake obviously moves along a reverse gradient of oxygen.

The different temperature coefficients, mentioned earlier (p. 66) for anion and cation uptake as found by Wanner (1948), certainly indicate separate absorption mechanisms. However, the conclusion that anion uptake is metabolically linked and cation uptake is of a physical nature (diffusion) would be too far-reaching, for van den Honert *et al.* (1955 *b*, *c*) found very high temperature coefficients for nitrate as well as for ammonium absorption at temperatures between 3° C and 6° C.

The theoretical value of 4 for the ratio salt uptake-salt respiration found by Robertson *et al.* (1948*b*), which seems to support Lundegårdh's hypothesis can, however, be interpreted in different ways. In Robertson's experiments, the same "salt respiration" was observed with various rates of salt uptake. From Robertson's Figures 1 and 2 (1948*b*), given here in Figures 11 and 12, it appears that in carrot tissue, salt uptake was doubled at the higher concentrations, while 'salt respiration" remained constant.

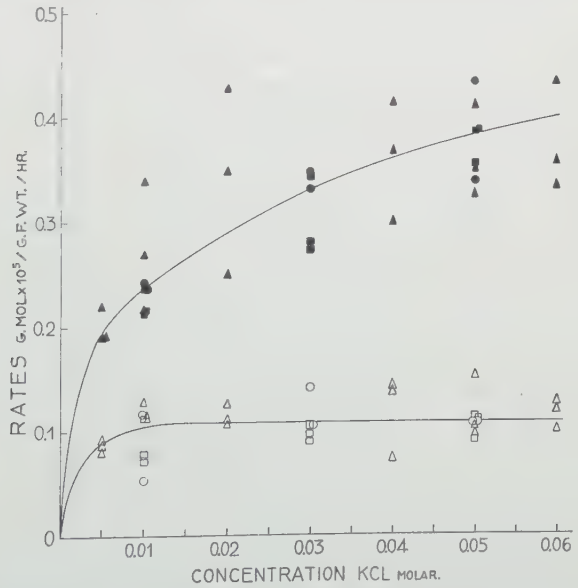


Fig. 11. Rates of salt respiration (open symbols) and salt accumulation (solid symbols) for carrot tissue in KCl solutions, after Robertson *et al.* (1948*b*, Fig. 2).

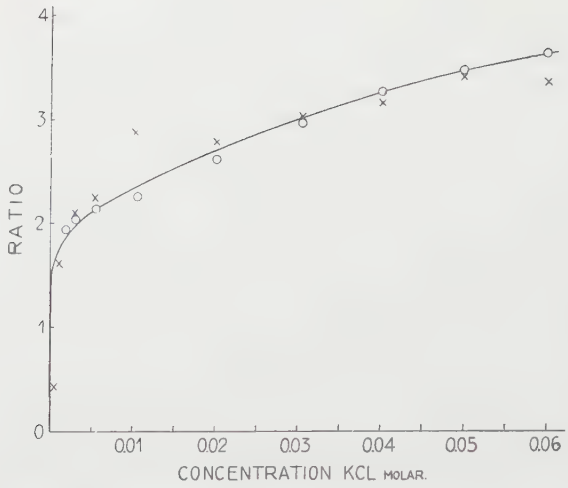


Fig. 12. The ratio between salt accumulation and salt respiration at different KCl concentrations, after Robertson *et al.* (1948*b*, Fig. 1).

A satisfactory explanation of this fact has not been given by Robertson. Actually, the theoretical value of 4 ultimately obtained for the ratio in question is due only to the increasing salt uptake without corresponding increase in respiration. In addition, Sutcliffe (1952) observed that in red beet root storage tissue K^+ uptake was greatly influenced by accompanying anions as well as by the internal K concentration of the cells (1952, Fig. 6). Nevertheless, in his experiments the "salt respiration" was stimulated to the same level in all cases (1952, Fig. 11). Even when salt uptake had ceased, the magnitude of "salt respiration" in his beet root remained the same as when accumulation was proceeding rapidly.

For an explanation of an increased salt uptake without a concomitant increase in "salt respiration", as reported by Robertson (1948*b*) and Sutcliffe (1952) in storage tissue, reference should be made to the work of van den Honert (1954). Van den Honert *et al.* found that the salt uptake in potato storage tissue slices did not show a saturation value at a low salt concentration as was observed in plant roots. In maize roots the uptake-concentration curve for NH_4^+ approached its maximal uptake value at a concentration of 10 p.p.m. NH_4^+, after which it increased only slightly with increasing NH_4^+ concentration. However, above 10 p.p.m. NH_4^+, the NH_4^+–ion uptake in potato storage tissue continued to increase markedly to about 400 p.p.m. NH_4^+, which gave an uptake-concentration curve very similar to that of Robertson (1948*b*). Van den Honert explained this "potato effect" by assuming a salt uptake by progressively deeper cell layers. Robertson's data can therefore be explained by the assumption that a primary salt uptake, mainly by the outer cell layers, has already initiated a maximum respiration response throughout the tissue, whereas at in-

creasing salt concentrations deeper cell layers share an ever-increasing part in salt uptake without a simultaneous increase in respiration.

Therefore, Robertson's ratio of 4 is rather arbitrary and its significance in favour of Lundegårdh's hypothesis should not be overestimated. Moreover, Lundegårdh (1949*b*, 1950*a*), repeating Robertson's experiments with wheat roots at the same high salt concentrations, failed to find this high ratio, and found instead an average of 0.40. Lundegårdh (1950 *a*, *b*) attributed this low ratio to energy, derived from respiration, required to transport "native anions" in the tissue and also to prevent leakage of ions from the roots. The latter explanation does not seem valid, because in cases with no salt uptake Sutcliffe (1952) found that if "salt respiration" was inhibited by cyanide, no leakage of ions occurred. In addition to these arguments, Robertson's (1948*b*) non-specific conductometric estimation method for salt uptake, as he himself states, can be referred only to "salt uptake" and "salt respiration" and not, as in Lundegårdh's conception, to "anion uptake" and "anion respiration".

There are additional characteristics of ion uptake which cannot be reconciled with Lundegårdh's hypothesis without further extensive assumptions or modifications. One of the most important of these is the entire inhibition of salt uptake by 2,4-dinitrophenol (DNP), while respiration remains unaffected or even becomes stimulated at certain DNP concentrations (*cf.* Robertson *et al.* 1950, 1951). The work of Loomis and Lipmann (1948) showed that DNP does not inhibit the cytochrome-cytochrome oxidase system, but rather interferes with the transfer of energy-rich phosphate. A direct connexion with the cytochrome system (see *e.g.* Lundegårdh's diagram, 1954, Fig. 6) is difficult to visualize here. In addition, phloridzin, which inhibits phosphorylations (*cf.* James, 1953, p. 218), also prevents phosphate uptake (*cf.* Helder, 1952).

Furthermore, Österlind (1951) found in *Scenedesmus quadricauda* that respiration was not inhibited by cyanide, while ion uptake was. Nance (1949) observed that salt uptake in barley roots was inhibited by 2,4-dichlorophenoxyacetic acid, which does not interfere with the cytochrome system. The inhibition of ion uptake by substances such as malonate (Machlis, 1944), a specific inhibitor of succinic acid dehydrogenase in the tricarboxylic acid cycle, evidently cannot be an argument against Lundegårdh's hypothesis, because they inhibit the production of H-atoms available for the cytochrome system.

James *et al.* (1952, 1953) demonstrated that in the roots of 7-day old barley plants it is the copper-containing ascorbic acid oxidase which acts as the principal terminal oxidase, and not the cytochrome oxidase. A substance such as diethyldithiocarbamate, which chelates copper and therefore inhibits ascorbic acid oxidase to a great extent, also inhibits ion absorption. Apparently in barley roots at this stage of development there is an inhibition of salt uptake which cannot be attributed to an inhibition of the cytochrome system (*cf.* Scott Russell, 1954).

Finally, reference should be made to the fact that although cyto-

chrome oxidase acts as principal terminal oxidase in many plant roots, an ion uptake exclusively coupled with a cytochrome system cannot be generalized: many strictly anaerobic organisms which entirely lack cytochromes (*cf.* STEPHENSON, 1950, p. 25) nevertheless have the capacity for ion absorption.

§ 2. DISCUSSION IN CONNEXION WITH THE PRESENT OBSERVATIONS

In the experiments with maize plants in continually-flowing water cultures (Chapter II) over a period of many days, a continuous and rapid NH_4^+–ion absorption was found, accompanied by an almost quantitative exchange with H^+ ions. We know from other experiments in static water cultures (VAN DEN HONERT *et al.*, 1955, unpublished) that the NH_4^+–ion uptake far exceeds the absorption of other ions. Therefore, the NH_4^+–ion uptake studied in the flowing-water cultures can be roughly characterized as the uptake of only NH_4^+ ions. In the opinion of the present author, this indicates an active uptake of a single cation. Other arguments, *e.g.* in connexion with the specificity of the first binding of NH_4^+ ions to the carrier system, are mentioned in Chapter IV.

LUNDEGÅRDH (1950 *a*, *b*) explained an excess cation absorption by the additional assumption that "native anions" (*i.e.* organic acid anions) are produced by the protoplasm and subsequently involved in the cytochrome system. ULRICH (1941), MACHLIS (1944), BURSTRÖM (1945), and JACOBSON *et al.*, (1950) observed a production of organic acids in response to excess cation uptake; conversely, organic acids disappeared when anions were accumulated in excess of cations. The latter authors (except Burström) see this phenomenon as a way by which the protoplasm maintains its anion-cation balance or electrostatic equilibrium with the medium. Therefore, it is questionable whether this increase or decrease of organic acids is related to the actual process of anion transport, as Lundegårdh claimed.

Experiments of VAN DEN HONERT *et al.* (1955, unpublished) showed that maize roots absorb ammonium and nitrate ions in the ratio of 3 : 1, at least if adapted to ammonium nutrition. Now, according to Lundegårdh's picture, the NH_4^+ uptake as measured in our experiments would be accompanied by a "native anion" transport three times as large as the transport of external anions in nitrate absorption.

Taking into consideration that nitrate is the most rapidly absorbed anion, it is clear that considerations like this lead to the assumption of a very large unknown component in anion transport. Therefore, it may be questioned what would be left of a direct relationship between external anion uptake and respiration as shown in LUNDEGÅRDH's original graphs (1933, Fig. 3; 1940, Fig. 19 and 21).

The experiments performed with excised maize roots (Chapter III) provide evidence in favour of a cation uptake mechanism and also of a "cation respiration". Non-deficient roots showed an appreciable NH_4^+–uptake but no respiratory response to ammonium salt addition. Only nitrogen-deficient roots gave an "ammonium ion respiration".

This is easily understandable as an ammonium-stimulated increase of nitrogen-deficient metabolic processes. The experiments with the NH_4^+-bearing exchange resins, which demonstrated that the uptake of a single cation caused an increase in respiration, seem to be particularly suggestive. There is no doubt that metabolism is associated with absorbed ions. This is supported by the evidence of variations in R.Q. values due to an excess cation or anion uptake as stated by Ulrich (1941), and the R.Q. differences in relation to ammonium or nitrate uptake observed in the present study.

It seems, therefore, that ion uptake and "salt respiration" are related in a more indirect way. Robertson *et al.* (1945 *a*, *b*) reported that "salt respiration" is initiated quickly (half-time value of 20 minutes), but lasts for 40 hours after subsequent transfer to distilled water. Although it may be possible to attribute the prolonged respiratory increase to internal salt transport, a more likely suggestion is that assimilated ions increase metabolism in general. The rôle and the ultimate fate of the ions in metabolism may have a profound influence on the nature and magnitude of the respiratory response.

Summarizing the conclusions of the present study, we may state that Lundegårdh's arguments in favour of the view that only anions are actively transported are not conclusive. The opinion that cations as well as anions are actively accumulated seems to be equally acceptable. However, one of Lundegårdh's arguments still remains to be discussed, *i.e.* the negative charge of the root surface which hampers anion uptake and facilitates cation uptake. Vervelde (1952, p. 46; 1953, p. 320) points out that this picture is incorrect, at least if the protoplasm membrane is considered to be a Donnan-equilibrium system. It is true that the entrance of anions is impeded by the drop in electrical potential at the surface, but there is at the same time a concentration fall in the opposite direction favouring the entrance of anions. The repellant action of the electrical charge is exactly balanced by the accelerating effect of the concentration fall, due to the equality of the electrochemical potential on either side of the surface membrane. Similar reasoning is valid for the uptake of cations. Their inward migration is favoured by the electrical potential difference, but retarded by the concentration difference. Hence, the negative charge of the root surface cannot be used as an argument in favour of Lundegårdh's anion respiration theory.

SUMMARY

(1) NH_4^+–ion uptake experiments are performed with intact maize plants in continuously-flowing water cultures. The NH_4^+ uptake-concentration curve fits best with a Langmuir adsorption equation. However, no strict saturation effect at the higher concentrations is observed. This can be explained by assuming an ever-increased participation of deeper cell layers in the NH_4^+–ion uptake process at the higher NH_4^+ concentrations. An agreement of the data with Langmuir's adsorption equation, however, does not imply that NH_4^+–ion uptake is a non-specific adsorption reaction. The same equation also holds for specific binding reactions such as enzyme reactions. In the present study, a first binding to specific carriers is proposed. Some

of the properties of the assumed carriers can be studied in intact living roots, because no measurable diffusion resistance exists between the place of first binding and the external medium. The uptake-concentration curve shows that the NH_4^+-ion uptake reaches its saturation value at about 10 p.p.m. NH_4^+. Its "half-value" concentration lies at 0.23 p.p.m. NH_4^+. This very low half-value concentration points to a great affinity between the carrier and NH_4^+ ions. No measurable difference in the rate of NH_4^+–ion uptake between pH = 6.0 and pH = 4.6 is observed. Therefore, between these limits, pH does not affect the carrier mechanism nor does the H^+ ion compete with the NH_4^+ ion for the same sites in the carrier. Arguments are put forward that the NH_4^+–ion uptake is a specific and active uptake, independent of the uptake of other species of ions.

(2) The well-known physiologically acid reaction caused by NH_4^+–ion absorption is quantitatively studied at a constant pH, again using the flowing culture solutions by which the excess of H^+ ions produced is continuously removed. It appears that in vigorously growing maize plants at low NH_4^+ concentrations (below 3 p.p.m.) a nearly quantitative exchange ratio of 1 : 1 of H^+ ions for NH_4^+ ions exists. At higher NH_4^+ concentrations, about 80 % H^+ ions are released for NH_4^+ ions absorbed. At still higher NH_4^+ concentrations (about 20 p.p.m.) the H^+–ion release tends to decrease gradually, probably due to an ever-increasing anion uptake which has not yet reached its saturation value. At pH = 4.6 the ratio of NH_4^+–ion uptake to H^+–ion release is not measurably different from that at pH 6.0. In older maize plants the H^+–ion release is markedly lower, because there exists an exchange of NH_4^+ ions of the medium for K^+ ions of the root. The K^+–ion release can be considerable. In addition, there is a lower NH_4^+–ion uptake due to a decreased capacity to synthesize in older plants. Arguments are put forward in favour of the view that not only NH_4^+–ion uptake, but also H^+–ion release, is linked to active metabolic processes.

(3) Respiratory changes due to NH_4^+–ion uptake are studied in excised maize roots with the standard Warburg manometric technique. These experiments—especially those with NH_4^+ bearing exchange resins—suggest a *cation-induced respiration, i.e.* a salt respiration caused solely by an active NH_4^+–ion uptake. Moreover, it is shown that the respiratory response depends primarily on a salt deficiency induced by protracted washing or starvation in distilled water, dilute salt solutions, or tap water. In fact, these responses can be obtained more specifically by making plants deficient in one particular mineral requirement: addition of the missing element—provided there is a sugar reserve—will immediately give a respiratory response.

Salt respiration can be initiated in roots suspended in relatively strong, one-element deficient, salt solutions by addition of the missing mineral element. Thus, a "low-salt" condition or a deficiency in a particular ion is the prerequisite for a particular related respiratory response. As both processes (metabolism as well as ion uptake) are dependent on a deficit of a specific ion, it seems probable that the connexion between salt uptake and salt respiration would be of an indirect nature, *i.e.* the salt respiration would not be directly linked with ion transport.

Nitrogen and phosphate starvation lead to considerable depression of the respiration rate, even when the carbohydrate content of the root tissue is high. Subsequent addition of nitrogen or phosphate salts markedly increases the respiration rate, while other ions do not affect the respiration to any degree. However, potassium starvation in maize roots produces an increase of respiration rate which is again specifically reduced by potassium salt addition. Once again arguments seem to be more in favour of a connexion of salt respiration with metabolism rather than with ion transport.

The opinion is expressed that the effect of an ion on respiration is in some way connected with the rôle and the fate of that particular ion in metabolism.

(4) Although the results of the present study do not strictly disprove Lundegårdh's "anion respiration" hypothesis, they can be completely understood by the assumption of an "active" cation uptake and "cation respiration". In fact, if no other results were known, this would be the obvious conclusion.

In principle, three different mechanisms of active salt uptake could be concieved: (a) an active anion uptake and a passive cation uptake, (b) an active cation uptake

and a passive anion uptake, (c) an active anion as well as an active cation uptake. A priori, no special preference can be given to any one of these mechanisms. On the basis of the evidence discussed in Chapter V, Lundegårdh preferred concept (a). The tendency of this thesis is, however, to emphasize that concept (c) is surely not less acceptable.

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THE AETIOLOGY OF SOME THRIPS GALLS FOUND ON LEAVES OF MALAYSIAN SCHEFFLERA SPECIES

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Introductory

The galls that are caused by thrips have not yet received the attention they deserve. This is perfectly comprehensible, because the few thrips-galls found in Europe have a very simple structure. The leaves are partly rolled inwards and poorly developed, and in case of heavy infection there is a general wilting of the whole plant, so that it looks diseased, (Grevillius, 1910). The infection takes place when the tissues are as yet little differentiated. They remain in this stage, and the infected organs show the symptom of hypoplasia.

The number of thrips-galls in the tropics of the old world is very large, particularly so in the Indo-Malay region, which has been best examined for galls. About one tenth of all zoocecidia are caused by these insects (Docters van Leeuwen–Reijnvaan, 1926). Some galls have a very simple structure, but others show a distinct tissue differentiation and also hyperplasia. Similar highly developed thrips-galls have also been found in Australia.

The subject of the present study are the galls caused by thrips on species of *Schefflera*. They have been chosen because there are particularities in their aetiology which have not been observed in the development of any other gall. The galls are common, and it is relatively easy to experiment with the insects.

The representatives of the genus *Schefflera* are erect or climbing shrubs; several grow epiphytically. The most common species is *Schefflera elliptica* and on this species too the thrips-galls are very common. The plant ascends, with its rather flaccid shoots high into the crown of the host-plant, the ends of the shoots often hanging down. As the growth goes on throughout the greater part of the year, the various stages in the development of the gall are often found close together. The plants are easily reproduced by cuttings, so that all the year round material can be at the student's disposal.

The galls

There are two kinds of thrips-galls on *Schefflera:*

1) Involute leaves. The two halves of the leaflet are incurved and meet along the main-nerve. The long, narrow gall-chamber formed in this way contains thrips in all stages of development.

I found this gall in Java on *Schefflera scandens* (Bl.) Vig. and in Sumatra and Java on unclassified species of *Schefflera*. These involute leaves, so commonly caused by thrips on other plants species too, will not be discussed here.

2) Horn galls. According to the species of *Schefflera* on which the galls develop, the upper and, rarely, the undersurface of the leaflets show either long, tubular or shorter and more irregularly formed galls.

Schefflera divaricata (Bl.) Koord. The galls are short and horn-like; they are 4–6 mm long and 1 to 1½ mm across. The position of the gall relative to the surface of the leaflets is often oblique. The opening is at the base. This gall was once collected at an altitude of 1600 m above sea-level near Tjinjiruan above Bandung, West Java.

Schefflera elliptica (Bl.) Harms. The gall is attached to the upper-surface of the leaflets and has a narrow, round aperture at the underside. These galls are tubular with a sharp or rounded top; they are often inclined towards the leaf-blade, and sometimes they are more or less spirally twisted. Sometimes they are thin and long, sometimes shorter and thicker, often with irregular, small knobs on the outer surface. The surface. is smooth, green, often covered with brown and particularly red stripes. (See the coloured pictures of these galls in Docters van Leeuwen–Reijnvaan, 1926, pl. III, fig. 6–9). The wall is fairly succulent and encloses a long, narrow gall-chamber, which extends almost from the opening to the top. The galls are from 10 to 30 mm long and from 2 to 4 mm across, see fig. 1.

Fig. 1. Two leaflets of *Schefflera elliptica* with many mature thrips-galls, x 3/4.

This gall is very common, and has been collected in many places in Sumatra, Java, Celebes, the Salajar Islands and Sumba. In Java it has been found from Bogor (Buitenzorg) in West Java to Djember in East Java. It is likely that it occurs wherever this common plant is present. The host-plant is found in Java from the plain up to the lower mountain regions.

Schefflera lucescens (Bl.) Vig. var. *grandifolia* (K. et V.). Bakhf. and var. *rigida* (Bl.) Bakhf. The galls which develop on this plant are also horn-like but the shape is different from that described for the gall on *Schefflera elliptica*. The horns are more irregular, often somewhat wider than high, and they sometimes give the impression of a vesicle. The surface of the galls is not smooth but scabrous, and the galls are often twisted and inclined obliquely towards the leaf-blade. Now the galls are green, and then again the colour is red or purple. The galls are from 2 to 10 mm high and from 2 to 5 mm thick; sometimes they are attached to the underside of the leaf with the opening at the upperside. In case of heavy infection the leaflets develop irregularly with deep incisions and contortions. Apart from these true galls one occasionally finds near the leaf-edge and particularly on the petioles long or short, curved, gibbose excrescences, which prove to be solid and are therefore no true galls. These excrescences may be up to 20 mm long and from 3 to 5 mm across. I collected these galls on the two varieties of *Schefflera lucescens* in various places in West Java. See figure 2.

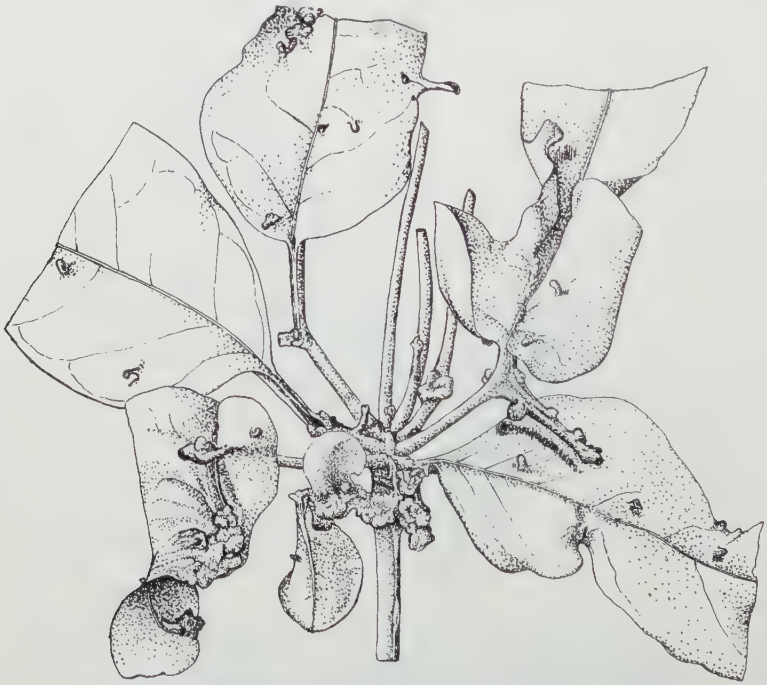

Fig. 2. A leaf of *Schefflera lucescens* var. *rigida* with many thrips-galls and solid outgrowths, x 3/4.

Schefflera polybotrya (Miq.) Vig. The galls on this plant look like those on *Schefflera elliptica*, but they are always green and the top is sharply pointed. Their position with regard to the leaf-blade is oblique; often they are pressed against it. The galls are from 5 to 30 mm long and from $1\frac{1}{2}$ to 2 mm across. The gall-chamber is very narrow and ends where the top contracts. As far as I know this gall was collected only on Mount Gedé in West Java. See fig. 3.

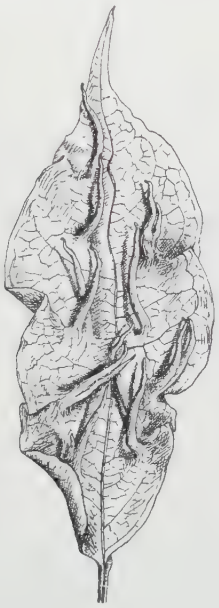

Fig. 3. A leaflet of *Schefflera polybotrya* with full-grown hrips- galls, × 3/4.

The gall-producing insects

The galls are caused by thrips, about 5 mm long and, like most species, coloured black. The insects leave the galls when full-grown; as most galls are inhabited by a few insects only, they are usually found empty. The insects are not very active and therefore they can easily be moved from one place to another. If the new place offers sufficient food, the insects as a rule quietly stay in the new place.

The only species described so far is that which lives in the galls on *Schefflera elliptica*. This insect is very similar to *Gynaikothrips chavicae* Zimm., which causes galls on the leaves of many species of *Piper*. The first insects from the *Schefflera* galls which I sent to the thrips specialist H. H. Karny (1912) in Vienna were indentified by him as *Gynaikothrips chavicae*. Afterwards when he had more material at his disposal he made them into a subspecies *hebtapleuri* (see Karny und Docters van Leeuwen–Reijnvaan, 1913, 109) and at a still later date (see Karny and Docters van Leeuwen–Reijnvaan, 1915) this

subspecies became a species *Gynaikothrips heptapleuri* Karny. PRIESNER (1926) who studied the larvae of thrips extensively, states that the larvae of *Gynaikothrips heptapleuri* are distinctly different from those of *G. chavicae*.

The full-grown insects are sometimes found inside the galls, but also fairly often living on the lower surface of very young leaflets of *Schefflera elliptica*. In this case they mostly sit without moving pressed against the main-nerve on the lower surface. However, in order to cause galls it is necessary that the insects move over the leaf surface.

ANATOMY OF THE GALL ON SCHEFFLERA ELLIPTICA (BL.) HARMS

The epidermis of a full-grown leaf consists of two layers of cells. The outer layer consists of small, low cells with a thin cuticle. Underneath lies a layer of larger, more isodiametric cells, which like those of the upper layer lack chloroplasts. Next come three layers of palisade parenchyma cells. The height of the cells of the upper layer is three times their thickness, while that of the second layer is twice their thickness and these cells, moreover, enclose small intercellular spaces. The height of the cells of the third layer is still smaller, about $1\frac{1}{2}$ times their thickness. They are also more rounded. The spongy parenchyma consists of 7 or 8 layers of cells with large intercellular spaces. The dimensions of these cells are larger in the longitudinal direction of the leaf than across, and their corners are rounded. The epidermis of the lower surface consists of a layer of cells that are similar to those of the upper surface; the cuticle is somewhat thicker. The vascular bundles are fairly small; laticiferous tubes are present.

At the time of the infection the small young leaf consists of from 13 to 14 layers of cells, which are not yet distinctly differentiated. The cells are small and closely pressed. The central mesophyll cells are still indistinct. The xylem elements and the cortical fibres are not yet lignified.

The first sign of the infection is a small spot, about 1 mm in diameter, at first transparent, but soon turning red. After a few days it develops into a small knob, which grows out into a tube on the upper side of the leaflet.

The wall of the young gall consists of as many layers as the young leaf, that is 13 or 14. The primordia of vascular bundles are soon visible. When the gall is about $2\frac{1}{2}$ mm high, the differentiation of the cells in the surrounding leaf-tissue has set in; epidermis and spongy parenchyma are distinct, but the spongy parenchyma does not yet contain any clear intercellular spaces.

The cells of the young galls are not rounded, but extended parallel to the axis of the gall. On the outside of the gall the cells are still arranged in regular layers, but towards the interior the structure is more irregular. Near the base of the gall, i.e. near the opening, the tissue grows out towards the interior so that the entrance becomes slightly narrowed. Many wood vessels are now clearly lignified. The vascular bundles anastomose in the top of the gall. The young galls

are coloured red. The red pigment, which is dissolved in the epidermis cells, disappears completely, or almost so, during the further development of the gall.

In the full-grown galls too the cells on the outside are arranged in layers. They are long and narrow. The cells situated more towards the interior are larger and reach their greatest dimension perpendicular to the axis. Locally this may lead to the development of small knobs on the interior wall of the gall-chamber. Then the gall-chamber is no longer a regular tube. In such places the vascular bundles join in the growth, less so the xylem, but to a greater extent the phloem and particularly the bast fibres. The latter may be strongly bent; see fig. 4.

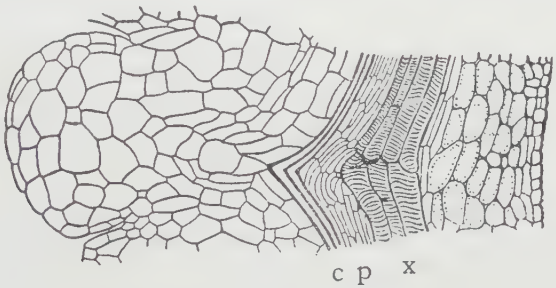

Fig. 4. Part of longitudinal section of the gall on *Schefflera elliptica*, c = cortical fibres; p = phloem and x = xylem.

The cuticle of the epidermis-cells which form the interior wall of the gall is on the whole well developed, but on the knobs it is thinner. The wall of the gall measures about 20 cells across; on the outside the cells have become more or less collenchymatous. The phloem is situated on the inside, the xylem on the outside of the gall. The xylem consists of smaller and larger groups of wood vessels, alternating with non-lignified elements. The bast fibres on the inside are best developed, less so on the outside, where they are not very much lignified either.

This gall is one of the most highly developed thrips-galls, a typical hyperplasia, therefore according to KÜSTER (1911) a prosoplasmatic gall.

AETIOLOGY

The development of a gall is due either to the influence of an egg or of a growing larva (Hymenoptera, Coleoptera, Lepidoptera and Diptera) or to the influence of a mature insect, as happens in the case of Aphididae, Coccidae and Phytoptidae. The gall arises in the place where the insect, the egg or the larva is in contact with the plant. As soon as the egg, the larva or the adhering insect is killed, the growth of the gall is arrested. The development of the *Schefflera* galls described above takes place in a different way.

The leaves of *Schefflera elleptica* are infected when they are still very young, although already expanded and coloured pale brown. The leaf-tissues are not yet differentiated, see the preceding chapter.

In the open air the following facts can be observed. On the leaves of a shoot where galls are found, a female thrips sits appressed against the main-nerve on the underside of a very young leaflet. The upper surface shows at first transparent spots, which soon turn red, probably a consequence of a wound reaction. Before long small excrescences develop from these red spots, which grow upwards; in the course of a few days these excrescences are from 2 to 3 mm high. The female is still living on the lower surface of the leaflet, but not constantly in the same place. The insect is seen again and again with its head entering some knob.

After about a week's time the gall has developed into a horn, and when this has become from 6 to 8 mm high, eggs can be found and soon afterwards young larvae. The latter grow rapidly, just like the gall; after one week the larva is mature and changes into an imago. The galls mostly contain but a small number of insects. The latter are imagoes and larvae in various stages of development; this shows that the eggs are not all deposited at the same time. When mature the insects soon leave the gall, so that most galls prove to be uninhabited.

These observations in the open air lead to the conclusion that all galls on a leaflet arise under the influence of a single female thrips or, when the leaflet is covered by a larger number of galls, probably of two females.

In March 1925 I undertook several experiments which proved that the above mentioned conclusion was right.

A great many cuttings with roots were planted in pots, one in each, and stripped of their leaves. Each cutting was covered by a wide glass tube. Attention was paid that no thrips were present either on the cutting or in the tube. Some male and some female thrips were then introduced in the tubes, and the results were awaited. The cuttings soon produced young leaflets, and as soon as the latter measured a few mm the females settled on the lower surface.

On March the 15th I introduced a female thrips into a tube. The leaves, however, were slightly too old; red spots arose, but they did not develop any further. In tubes with younger leaves the galls developed in great numbers.

On March the 20th a female was brought into a tube containing a cutting with very young leaves. This female settled immediately on the lower surface of a leaflet, and as early as the 21th of March transparent spots were visible. These spots soon turned red and on the 22nd of March small excrescences of about $\frac{1}{2}$ mm could be found. Next day the young galls measured from 2 to 3 mm. The female insect was seen first here then there penetrating with the front part of the body into the young galls. The galls grew regularly, and on March the 26th some of the galls contained eggs. The female thrips now entered a full-grown gall with her whole body, left it and entered another gall and so on. As soon as the female was taken away the growth of the galls immediately stopped.

A second experiment was made from the 22th March onwards; on

March 26th the galls measured 3 mm. At this moment the female was taken away and the galls did not develop any further.

The two experiments described above and several similar ones lead to the conclusion that all galls on a leaflet arise under the influence of one female thrips; also that the growth of the gall at whatever stage of its development stops when the female is taken away. Only when the galls contain larvae, growth continues without the presence of a female. At that time, however, the galls have practically reached maturity.

A cutting of *Schefflera elliptica* which only bore the petioles of very young leaflets, was covered with a tube containing some thrips. The insects punctured the petioles and took some food; soon after red spots were seen, but no excrescences developed. Further experiments yielded the same result.

Another species of *Schefflera*, *S. lucencens* was also subjected to experiments, because they bore, apart from normal galls, excrescences on the leaf-edges and on the petioles which proved to be solid. The leaves of this species are much larger than those of *Schefflera elliptica*. The leaflets of this very robust species are also infected when still very young. Mostly more than one female thrips was found on the lower surface of the leaflets, and these were often covered with dozens of galls. Cuttings of var. *rigida* were subjected to the following experiments. Female thrips were introduced into a tube containing cuttings, the young leaflets of which had been removed. Only the petioles were left. The thrips sucked the petioles and remained alive; contrary to what had happened in the other species of *Schefflera* we now found that

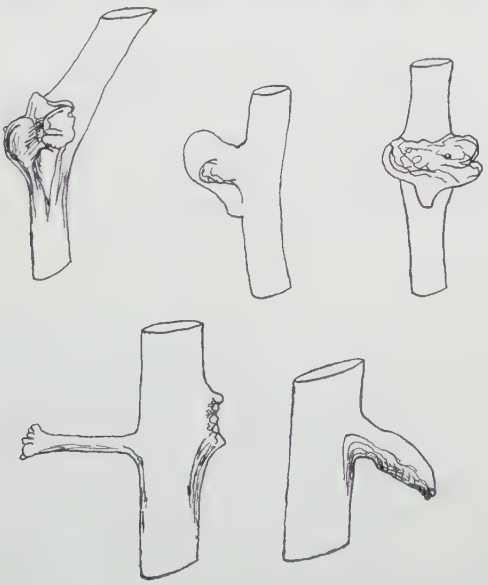

Fig. 5. Solid outgrowths which arose during experiments on the petioles of leaflets of *Schefflera lucescens* var. *rigida*, × 2.

after several days excrescences of various shape developed on the petioles. Sometimes they were clavellate appendages, while at other times they looked like cancerous outgrowths. All these excrescences were solid. Some of the excrescences obtained during these experiments are illustrated in fig. 5.

In the description of the gall on *Schefflera lucencens* on page 82 it is stated how by the side of the normal galls on the leaf-edges and on the petioles excrescences of various forms occur. The above-mentioned experiment shows that these excrescences arise under the influence of the sucking thrips. I have not been able to state whether the excrescences arise after one single puncture or whether the insects must puncture the plant several times in the same place.

Küster (1911) calls such excrescences "verirrte Gallen", that is stray galls, although the term gall is stricktly speaking not correct. Küster is of opinion that such stray galls are theoretically of great importance.

Conclusions

The most important result of the observations and experiments described above is no doubt the realisation that on a *Schefflera* leaflet several galls arise under the influence of a single female thrips. The thrips punctures the very young leaflets on the lower surface in various places, and in these same places the galls are formed. When the latter have reached a certain stage of development the female begins to deposit eggs in them. It was also demonstrated that the growth of the gall stops as soon as the female thrips is taken away.

We are justified in assuming that the sucking thrips introduces substances, probably from the salivary glands, into the young undifferentiated leaf-tissue so that the development of the cells is affected. The nature of only a part of these substances is known. Generally speaking one may say that these galls arise under the influence of chemical substances. In this case the gall is a chemomorphosis. This term has indeed often been used in discussions on the aetiology of other galls. The term in itself, however, implies but little, and does not throw any light on the processes taking place in the development of the infected cells.

During the last few decades of last century and of the beginning of this century many extensive articles have been published on the aetiology of galls. A good review is given in Küster's book (1911). Zweigelt (1931) examined the structure and development of some galls caused by plant-lice on *Ulmus;* he extensively discusses the problems turning up during the growth of a gall. Beijerinck too has devoted many studies to this subject. Werner Magnus (1914) has written a very clear review. Among other things he points out that Fitting in his examination of the simple postfloral changes of the flowers of some orchids found that several factors play a part in this process; if the galls were really to be looked upon as chemomorphoses, varied influences must be attributed to chemical substances secreted by gall-

producing animals or their larvae. If we realize how many factors play a part in the development of a normal cell, it is easy to understand that it must be at this stage impossible to analyze the factors leading to the formation of a gall. I therefore restricted this article to a short description of the problems of gall-forming.

Werner Magnus (1914) ends his discussion with the following words (translated): "Before we can even think of solving the gall problem, many extensive investigations and experiments into the history of their development will be necessary in order to explain some minor details of the aetiology of the galls. We have had enough hypotheses; let us at last see facts".

I fully agree with his words.

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BROMELIACEAE OF SURINAME

BY

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(received December 12th, 1955)

In the course of preparing a treatment of the *Bromeliaceae* for the "Flora of Suriname" four new species have been encountered and are here recorded for the first time.

Pitcairnia Geyskesii L. B. Smith, **nov. spec.**

A *Pitcairnia nuda* Baker, cui affinis, laminis foliorum subtus dense lepidotis, floribus minoribus differt.

Planta acaulis, verisimiliter metralis vel ultra sed e fragmentis solum cognita. Folia plurima, 4 dm longa, vaginis suborbicularibus, parte occulta atro-castanea glabraque, tertia parte suprema laminam simulante, laminis angustissime triangularibus, basi nullo modo attenuatis, longe acuminatis, pungentibus, 1 cm latis, supra glabris, subtus lepidibus magnis cinereis adpressis omnino indutis, spinis patentibus brunneis 4 mm longis laxe armatis. Scapus ignotus. Inflorescentia laxe bipinnata, glabra. Bracteae primariae triangulares, quam bases steriles

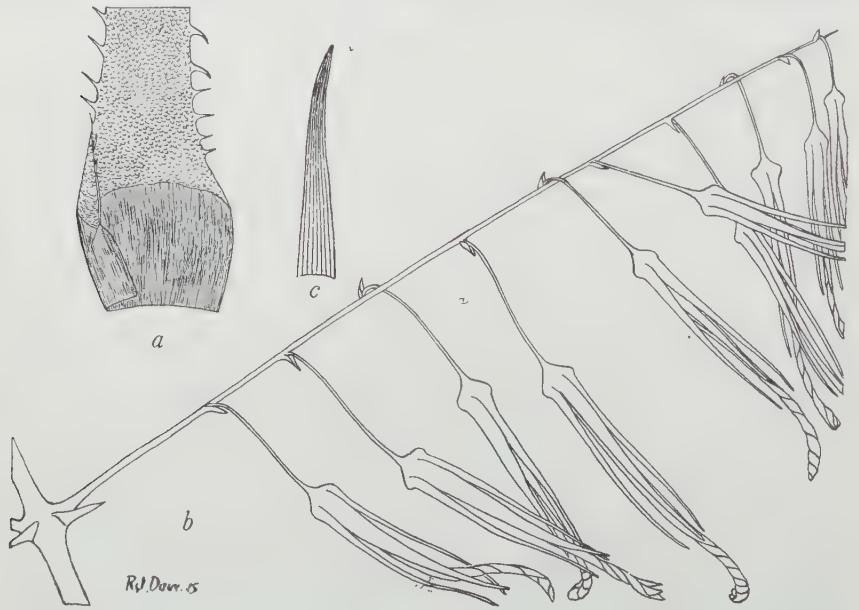

Fig. 1. *a*: *Pitcairnia Geyskesii* L. B. Smith, base of leaf 1; b: branch of inflorescence 1; c: sepal 1.

ramorum breviores, supremis integris. Rami patente-adscendentes, 4 dm longi, gracillimi. Bracteae florigerae late ellipticae, acutae, 4 mm longae. Pedicelli gracillimi, 2 cm longi. Flores pendentes, secundi. Sepala anguste triangulares, abrupte acuta, 30 mm longa, basi obscure carinata. Petala 40 mm longa, nuda. Stamina exserta. Ovarium 2/3 superum. Capsula dehiscens; seminibus alatis.

Suriname: Rocky slopes, Temomairem (Geyskes 10, Typus in Herb. Rheno-trai. fl. et fr. m. Julio).

Bromelia alta L. B. Smith, **nov. spec.**

Statura maxima et bracteis primariis florigerisque amplis facile distinguenda.

Planta florigera ad 2 m alta vel ultra. Folia multa, suberecta, 3–4 m longa; vaginis subtriangularibus, atro-castaneis, lepidibus linearibus brunneis indutis; laminis linearibus, acuminatis, pungentibus, verisimiliter ad basin versus paulo attenuatis, 7 cm latis, late canaliculatis, supra glabris, subtus pallide lepidotis, spinis latis curvatis applanatis 6 mm longis laxe serratis. Scapus validus, dense brunneo-lepidotus. Scapi bracteae fragilae; laminis mox perditis; vaginis imbricatis, subellipticis, ultra 12 cm longis, serrulatis, brunneo-lepidotis. Inflorescentia dense cylindrica, obtusa, 25 cm longa, 7 cm diametro, petalis inclusis brunneo-lepidota. Bracteae primariae erectae, breviter laminatae; vaginis suborbiculares, ramos breves dense florigeros magna ex parte obtegentes. Bracteae florigerae oblongae, acutae, 3–4 cm longae, conduplicatae, serrulatae. Flores obscure pedicellati. Sepala subtriangularia, 20 24 mm longa, libera, tenuia, fragilia. Petala 27 mm longa; laminis purpureo-maculatis; tubo filamentorum 5 mm longus. Fructus ellipsoideus 2 cm longus.

Suriname: Terrestrial, Paramaribo, Saramacca R., near sea level (M. B. Foster 2378, Typus in U. S. Nat. Herb., fr. Oct.); very abundant on top of high sand ridge in forest, Perica River, Commewijne District (Lindeman n. 5316, fl. Jan.).

Bromelia Fosteriana L. B. Smith, **nov. spec.**

A *Bromelia Morreniana* (Regel) Mez foliis haud distincte petiolatis, et a *B. agavifolia* Brongn. sepalis alte connatis, petalis dense brunneo-lepidotis differt.

Breviter caulescens, basi propaginibus multis erectis sese procreans. Folia multa, patentes, 9–12 dm longa; vaginis oblongis, 4 cm latis, brunneis, lepidibus magnis linearibus brunneis indutis; laminis sublinearibus, longe acuminatis, ad basin versus attenuatis, dein in parte suborbiculari abrupte dilatatis, 5 cm latis, medio late canaliculatis, brunneo-lepidotis, supra mox glabris, spinis 1.5 mm longis laxe serratis. Inflorescentia pauciflora, dense corymbosa, 5 cm diametro, inter folia intima nidulans, petalis ipsis inclusis dense brunneo-lepidota. Bracteae primariae subfoliaceae; laminis parvis; vaginis sepala aequantibus. Bracteae florigerae oblongae, ovarium multo superantes, serrulatis, ex sicco tenues fragilesque. Flores obscure pedicellati. Sepala oblonga, obtusa, cucullata, 21 mm longa, ad medium connata, serrulata. Petala lineares, obtusa, cucullata, 37 mm longa, stamina multo

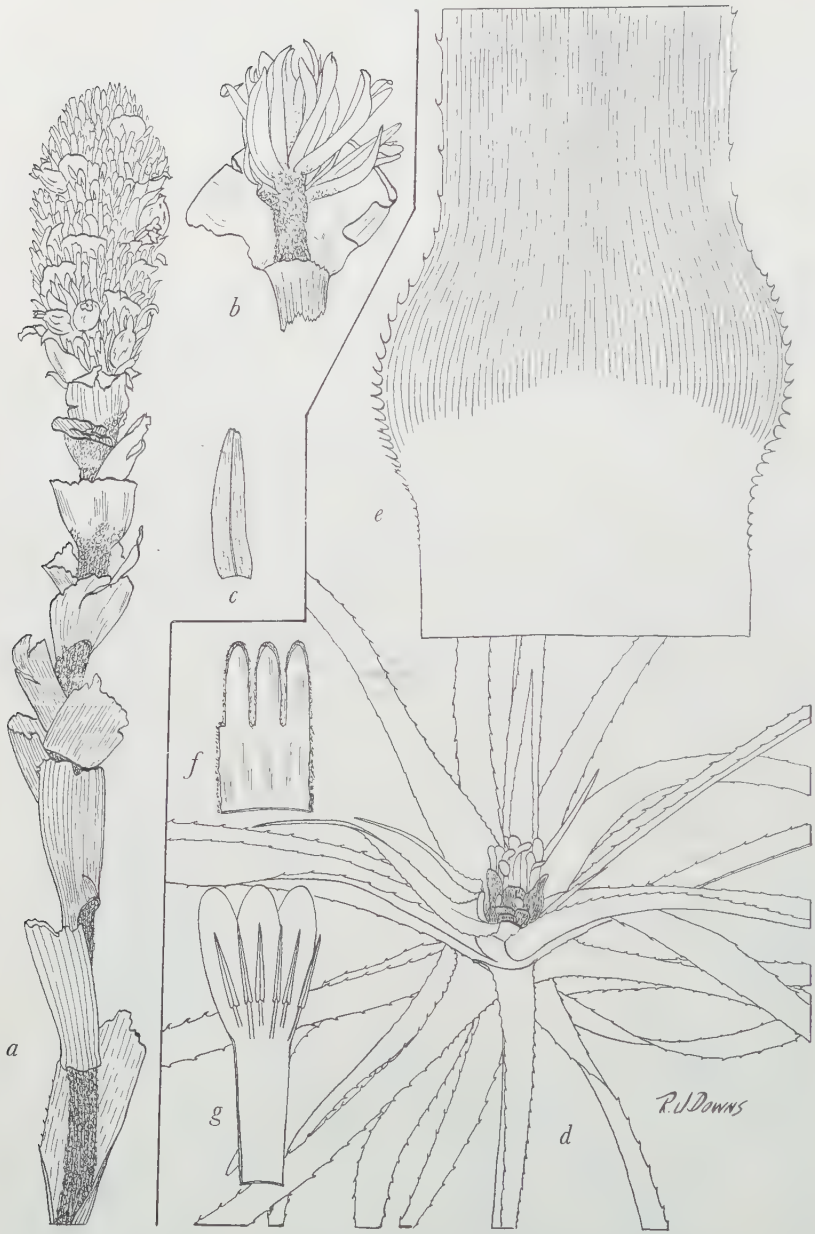

Fig. 2. a: *Bromelia alta* L. B. Smith, upper scape and inflorescence 1/4; b: branch and sheath of primary bract 1/2; c: sepal 1; d: *Bromelia Fosteriana* L. B. Smith, plant (after photograph by M. B. Foster); e: base of leaf-blade 1; f: ventral side of sepals 1; g: petals and stamens 1.

superantes. Tubus filamentorum 17 mm longus. Ovarium subcylindricum, ad 3 cm longum.

Suriname: Terrestrial, dense moist forest, Paramaribo (M. B. Foster 2389, Typus in U. S. Nat. Herb. fr. m. Oct.).

Gravisia Lanjouwii L. B. Smith, **nov. spec.**

A *Gravisia aquilega* (Salisb.) Mez et *G. capitata* (Schult.) L. B. Smith floribus spicatis, et a *G. Constantinii* Mez bracteis florigeris magnis differt.

Fig. 3 a: *Gravisia Lanjouwii* L. B. Smith, primary bract and branch of inflorescence 1; b: expanded sepal 1; c: longitudinal section of flower 2.

Planta ultra 1.2 m alta. Folia ultra 6 dm longa, lepidibus appressis albis centro brunneis induta; vaginis subellipticis, ca. 2 dm longis; laminis ligulatis, late acutis et pungente cuspidatis, 6 cm latis, spinis atris 3 mm longis laxe serratis. Scapus validus, albo-flocculosus, mox glaber. Scapi bracteae imbricatae, magnae, ellipticae, acutae, integrae, dense pallido-lepidotae; supremis roseis. Inflorescentia 2–3–pinnata, basi laxa, subcylindrica, 3 dm longa; axibus flocculosis. Bracteae primariae eas scapi simulantes, ramos infimos superantes. Rami patentes; axibus florigeris elongatis, floribus spicatis. Bracteae florigerae late ovatae, mucronatae, 15 mm longae, ovarium superantes, integrae, late convexae, ad apicem versus carinatae, prominente nervatae, conspicue hyalino-marginatae, glabrae. Sepala asymmetrica cum ala semiobovata, 12 mm longa mucrone 1 mm longo incluso, basi ad 2 mm connata. Petala ligulata, 15 mm longa.

Suriname: On granite flat near Voltzberg (Lanjouw 874, Typus in Herb. Rhenotrai. fl. m. Sept.).

ACTA BOTANICA NEERLANDICA, Vol. 5 (1), 1956

NEW TARAXACA FROM THE NETHERLANDS

BY

J. L. VAN SOEST

(received December 16th, 1955)

1. **Taraxacum agaurum** v. Soest **nov. spec.**

T. laetum non Dahlstedt, 1905, Botaniska Notiser p. 169; v. Soest, 1939, Ned. Kruidk. Arch. 49: p. 228, cum icon. (pro T. laeto!).
T. Soesteanum Haglund (manuscr. 1943), nomen nudum.

Planta sat robusta 10–25 cm alta basi fragmentis foliorum vetustiorum incrassata.

Folia numerosa vulgo terrae adpressa laete gramineo-viridia parce araneoso-pilosa nervo mediano pallido, petiolis brevis subalatis pallide viridibus. Folia exteriora lanceolata paulo lobata obtusa. Folia interiora ± lingulata utrinque ca 6-loba; lobi laterales triangulares saepe angusti acuti ± integri, interlobiis sublatis saepe longe grosse dentatis; lobus terminalis deltoideus vel hastatus obtusus vel subacutus, lobulis lateralibus saepe elongatis lobis lateralibus vel dentibus interlobii similibus.

Scapi numerosi subcrassi subrubescentes erecti folia superantes sub involucro ± araneosi.

Involucrum mediocre 1,5 cm longum, 1,5 cm latum crassiusculum viride. Squamae exteriores adpressae ovatae 8 mm longae late albo- vel roseolo-marginatae corniculis conspicuis instructae; squamae interiores late lineares subanguste marginatae apicibus corniculatae.

Calathium planum radians ad 3,5 cm diametro saturate luteum; ligulae marginales planae extus stria cano-violacea notatae. Antherae polliniferae; stylus et stigmata lutei. Floret vere, ab ineunte usque ad medium mensem maium.

Achenium obscure badio-violaceum 4 mm longum (pyramidi inclusa) superne argute erecte spinulosum ceterum laeve, in pyramiden cylindricam 1 mm longam laevam abrupte abiens; rostrum 9 mm longum, pappus albus 5 mm longus.

E sectione *Erythrospermorum* Dt.

Typus: Waalsdorp, pr. 's Gravenhage, in dunis maritimis, 29. IV. 1948, v. Soest (herb.).

Originally I identified this species as "cfr. *T. laetum* Dt.", and after consulting Christiansen I recorded it as such in Ned. Kruidk. Arch., Haglund, who received dried material from me, wrote me (1. V. 1943) in answer to my request for critical observation; "den är obeskriven

ännu. Jag har tillåtit mig kalla densamma *T. Soesteanum* m.". I visited Stockholm in 1948, and discussed this species with him, and in 1949 we together visited the type locality. After a long and serious illness Dr Haglund died in 1955, unable to describe and publish the new species. In the meantime I distributed it to several botanical collections under the name proposed by Haglund, as "nomen provis". I now prefer to drop this name, which is but a "nomen nudum", and I describe the species as *T. agaurum* m.

T. agaurum is allied to *T. laetum*, with which it has in common the yellow styles, the green petioles, the big warts on the phyllaries; it differs from it by its taller appearance, the larger heads, a more saturate colour of the flowers, bigger fruits and a slightly different leaf form. It is flowering nearly two weeks later than the other *Erythrosperma* in the same region.

Distribution: T. agaurum covers only a very small area of the Dune district of Holland; I never have seen plants from other countries, and I have studied collections from nearly everywhere in Western and Northern Europe. In this small area—a small stretch of seadunes along the North sea coast—it is very common in the *Violeto-Corynephoretum dunense* and in the *Tortuleto-Phleetum arenarii*, and it is often accompanied by *T. obliquum*.

Very common near the Hague from Loosduinen and Kijkduin to Wassenaar, see also Ned. Kruidk. Arch l.c.; furthermore: Noordwijk, de Jongh and v. Soest (herb.).

Variability:

f. *tubulosa* v. Soest: ligulis breves tubulosis, marginalibus extus purpurascentibus.

Dunes of Waalsdorp, 1935 v. Soest (herb.); also Kijfhoek and Wassenaar.

f. *colorata* v. Soest: petiolis nervoque mediano pro parte purpurascentibus.

's Gravenhage, 1936, v. Soest (herb.); also Waalsdorp and Meyendel.

2. **Taraxacum dunense** v. Soest **nov. spec.**

T. tenuilobum auct. non Dahlstedt, 1905, Botaniska Notiser p. 167; v. Soest, 1939, Ned. Kruidk. Arch. 49: p. 233, cum icon. (pro *T. tenuilobo*!)

Planta 5–25 cm alta gracilis basi araneoso-pilosa fragmentis foliorum vetustiorum incrassata.

Folia ± glabra gramineo-viridia saepe p.p. atro-purpureo-margi-, nata nervo mediano petiolisque intense purpureis. Folia exteriora lanceolata dentata vel paulo lobata; lobus terminalis ± deltoideus obtusus. Folia interiora multilobata ad 20 cm longa (petiolo incluso); lobi laterales ± irrigulariter distributi falcati p.p. lineares vel lingulati acuti, p.p. longe anguste dentati; interlobii perangusti valde et longe dentati, dentibus p.p. lobiformibus; lobus terminalis ± tripartitus, lobulo apicali lanceolato subacuto, lobulis lateralibus erecto-patentibus, omnibus integris.

Scapi purpurei basin versus intense vinosi glabrescentes.

Involucrum obscure viride 11–13 mm longum, ca 10 mm latum. Squamae exteriores laxe adpressae apice ± patentes ovato-lanceolatae 6–7 mm longae ca 3 mm latae anguste sed conspicue albido- vel purpureo-marginatae sub apice purpureae corniculatae vel callosae. Squamae interiores late lineares p. max. p. anguste marginatae apice purpureae corniculatae.

Calathium laete luteum planum radians ca 2,5 cm diametro. Ligulae marginales extus stria fusco-purpurea notatae. Antherae polliniferae; stylus et stigmata fusco-virescentes. Floret vere.

Achenium badio-rubrum 4 mm longum (pyramidi exclusa) superne argute spinulosum ceterum tuberculatum—basi laeve, in pyramiden (spinulis saepe praeditam) cylindricam 0,8 mm longam subsensim abiens; rostrum 8 mm longum; pappus niveus 5,5 mm longus.

E sectione *Erythrospermorum* Dt.

Typus: Scheveningsche Boschjes pr. 's Gravenhage, in dunis maritimis, 1922 v. Soest (herb.).

T. dunense is closely related to *T. tenuilobum* Dt., differing however by the shorter and broader, adpressed outer phyllaries of the involucre, by the shorter endlobes of the leaves and by longer fruit and rostrum, the first of a darker colour. In general, the red colour of the petioles and nerves is deeper, and the scapes are thinner and less arachnoid.

This species has been wrongly interpreted by Christiansen (1938) and by me in Ned. Kruidk. Arch. l.c. Afterwards Haglund and I discussed it, and we came to the conclusion that it was a new species.

Distribution: only along the North sea coast in the dunes, often accompanied by *Salix repens* ssp. *arenaria* (L.) And., *Viola coniophila* Wittr. a.s.o.

Very common in the dunes of Wassenaar and the Hague (Ned. Kruidk. Arch. l.c.); furthermore: dunes near Zandvoort, J. Prins (herb. Gorter). It is also common in the Flemish dunes (v. Soest, Bull. Soc. Bot. Roy. Belg., 1956).

3. **Taraxacum frisicum** v. Soest **nov. spec.**; fig. 1.

Planta ca 15–25 cm alta subglabra.

Folia luteo-viridia lanceolata 10–15 cm longa (petiolo angusto rubro incluso), interiora valde lobata; lobi laterales (utrinque 3) triangulares interdum ± retroversi acuminati acutissimi integri vel (inferiores) denticulati, interlobiis ± latis integris; lobus terminalis elongatus ad 3,5 cm longus ad 1 cm latus subobtusus vel obtusus saepe sublobatus, lobulis lateralibus acuminatis acutis ± retroversis.

Scapi glabri rubri ad anthesin erecti vel plerumque sigmatoidei.

Calathium mediocre paulo radians. Involucrum obscure viride interdum purpurascens; squamae exteriores adpressae ovatae 4–7 mm longae ad 4 mm latae in apicem obtusam acuminatae sublate marginatae ± erosae vel ciliolatae; squamae interiores ad 15 mm longae late lineares. Ligulae ± saturate luteae, marginales extus stria obscure rubro-violacea notatae. Antherae polliniferae; stylus et stigmata sordide lutei. Floret vere.

Fig. 1

Fig. 2

Fig. 3

Achenium pallide stramineum angustum (ad 1 mm latum) ad 4 mm longum (pyramidi inclusa) superne subdense ± patente spinulosum (spinulis ¼ mm longis), ceterum tuberculatum—basi laeve, in pyramiden cylindricam 0.8–1,0 mm longam laevam subabrupte abiens. Rostrum ca 8 mm, pappus albus 5–6 mm.

E sectione *Palustrium* Dt.

Typus: Akkerwoude, Lytsen (Friesland), in blue-grass pasture, 1953 v. d. Ploeg (herb., herb. v. S.).

T. frisicum is allied to *T. limnanthes* Hagl., which is lacking pollen and has broader-margined outer phyllaries in the involucre; the petioles of *T. frisicum* are deeper coloured. In small individuals the leaf form may resemble that of *T. limnanthes*.

T. frisicum is also related to *T. balticiforme* Dt.; the latter, however, has bigger, more acute, less acuminate and very broadly margined outer phyllaries; their leaf form is much alike.

Small individuals of *T. copidophyllum* Dt. sometimes imitate *T. frisicum*, but they specifically differ from it by the characteristic Palustria involucre of *T. frisicum*, which, moreover, has glabrous scapes.

Distribution: in peat-bog country, accompanied by *Hierochloë odorata* (L.) Whlnbg., *Viola palustris* L., *Taraxacum limnanthes* Hagl., *T. adami* Claire a.s.o., mostly in the *Caricion fuscae*. Only in Friesland.

Akkerwoude; Lytsen and Heechfinne; Eernewoude: Alde Feanen, Compagnie, Fokkesloot, Princehof; collected by v. Brakel, Franke (herb.), v.d. Ploeg (herb.), Vlieger and v. Soest (herb.).

4. **Taraxacum johannis-jansenii** v. Soest **nov. spec.**; fig. 2.

Planta mediocris ad 15 cm alta glabrescens.

Folia numerosa lobata subobscure viridia nervo mediano pallido, petiolis alatis pallidis. Folia exteriora minus lobata, interiora utrinque 4–5 loba ± crispata; lobi laterales saepe ascendentes hamati margine superiore convexo interdum 1 (-2) dente grosso muniti, saepe (lobi inferiori) minute denticulati, margine inferiore valde concavo raro 1 dente muniti; interlobiis interdum sublongis, saepe grosse dentatis; lobus terminalis hastatus subacutus, lobulis lateralibus elongatis acutis.

Scapi subglabri, folia breviter superantes.

Involucrum crassiusculum basi rotundatum obscure viride. Squamae exteriores laxe adpressae ovatae ca 6 mm longae 3 mm latae inaequilaterae obtusae conspicue (interdum late) albo- vel purpureo-marginatae, apicibus interdum purpureae saepe inconspicue callosae; squamae interiores late lineares (ad 2,5 mm latae) membranaceo-marginatae, apicibus interdum inconspicue callosae.

Calathium paulo radians ad 2,5 cm diametro obscure luteum. Ligulae marginales angustae ± planae extus stria cano-violacea ornatae. Antherae ± parce polliniferae; stylus et stigmata obscure fusco-virescentes, subnigri. Floret vere.

Achenium stramineum 4 mm longum (pyramidi inclusa) ad 1,2 mm latum superne ± breve spinulosum ceterum ± rugosum—basi laeve,

in pyramiden conicam 0,4 mm longam subsensim abiens; rostrum 8 mm, pappus albus 5 mm.

E sectione *Vulgarium* Dt.?

Typus: Middelaar, Johan Jansen, 20. V. 1941 (herb. v. S.).

This is an interesting species, more or less intermediate between *Spectabilia* and *Vulgaria*; in this respect it resembles *T. hygrophilum*, described below, and some other species of northern Europe. Whereas the Spectabilia have large fruits, *T. johannis-jansenii* has small ones. I think, it must be placed in the *Vulgaria* group, though it shows no direct affinity to any species of this section.

Distribution: common only in a small area near the type locality in the surroundings of Nijmegen, growing in acid and humid pastures.

Middelaar, Mook and Mooksche Broek, Plasmolen, Wychen, Orthen; collected by Joh. Jansen, J. Kern and Th. Reichgelt (herb. L., Roy. Netherl. Bot. Soc., a.s.o.).

5. **Taraxacum hygrophilum** van Soest **nov. spec.**; fig. 3.

Planta humilis ca 7–10 cm alta.

Folia 4–5 cm longa (petiolo brevi alato pallido incluso) ovato-lanceolata utrinque 2-vel 3-loba glabra nervo mediano pallido; lobi laterales ovato-triangulari dorso convexo integri vel paulo denticulati, margine inferiore integri; interlobiis subnullis ± crispis; lobus terminalis brevis semi-orbicularis vel hastatus.

Scapi 1–2 sub involucro araneösi.

Involucrum mediocre 10–12 mm longum 7–9 mm latum basi truncatum obscure viride. Squamae exteriores adpressae ovato-lanceolatae ad 5 mm longae ad 2,5 mm latae albo- vel roseolo-marginatae ciliolatae, apicibus interdum purpureae laeves; squamae interiores late lineares.

Calathium paulo radians ad 2,5 cm diametro obscure luteum. Ligulae marginales planae extus stria cano-violacea notatae. Antherae polliniferae; stylus et stigmata obscuri. Floret vere.

Achenium parvum 2,8 mm longum (pyramidi inclusa) ca 1 mm latum brunnescens superne squamulis tricuspidatis parvis praeditum, ceterum laeve, in pyramiden conicam 0,45 mm longam subabrupte abiens; rostrum subbreve 3 mm; pappus albus 4–4,5 mm.

Inter sectiones *Spectabilium* Dt. et *Vulgarium* Dt.?

Typus: Veenkampen, Grebbevallei, in acid peat bog pasture, v. Soest, 27. V. 1941 (herb.), accompanied by *T. nordstedtii* Dt. and *T. adami* Claire.

This species is closely related to *T. johannis-jansenii;* the heads are similar, a little bit smaller and more truncate at the base; the outer phyllaries are less broad; the fruits are smaller. The leaves are different, but in younger individuals the difference is small.

In general aspect it shows a great affinity to the Spectabilia; the fruits, however, are extra-ordinary small.

The real *Spectabilia* with big fruits are tetraploid (2 n = 32), *T.*

nordstedtii even is hexaploid. The *Vulgaria* all seem to be triploid. *T. praestans* Lb. f., considered an intermediate species, is triploid; it should be of interest to examine the chromosome number of *T. hygrophilum* and *T. johannis-jansenii*.

T. hygrophilum seems to be related to *T. hibernicum* Hagl., described from Ireland; this, however, has much larger fruits.

Distribution: in humid grassfields, especially in blue-grass pastures on acid soil, here and there in the Netherlands.

Grebbe valley (see above); Nieuw-Lekkerland, Haglund, Kloos (herb.) and v. Soest (herb.); Ulvenhout, v. Soest (herb.).

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FLORISTISCHE NOTITIES 1-18

DOOR

S. J. VAN OOSTSTROOM EN TH. J. REICHGELT

(*Rijksherbarium, Leiden*)

(*received January 18th, 1956*)

Met deze eerste serie Floristische Notities beginnen wij een reeks van korte mededelingen op het gebied van de Nederlandse floristiek, die bedoeld is als een voortzetting van de „Aanwinsten van de Nederlandse Flora" en de „Floristische Aanteekeningen", die vele jaren lang resp. door Kloos en door Jansen en Wachter in het Nederlandsch Kruidkundig Archief werden gepubliceerd.

Wij willen ons hierin niet strikt beperken tot het signaleren van voor Nederland nieuwe indigenen en nieuwe adventieven, zoals dat in Kloos' Aanwinsten geschiedde. Onze bedoeling is ook om korte, critische beschouwingen te geven betreffende reeds eerder hier te lande aangetroffen taxa, gebaseerd op eigen onderzoek of op in het buitenland verschenen literatuur. Wij hopen dat deze Notities in de toekomst van nut kunnen zijn als bouwstoffen voor de Flora Neerlandica.

De Floristische Notities zullen op ongeregelde tijden, maar toch minstens één maal per jaar verschijnen. De behandelde onderwerpen worden doorlopend genummerd, waardoor raadplegen en citeren vergemakkelijkt wordt. De gebruikte afkortingen voor namen van herbaria, waar zich het vermelde materiaal bevindt, zijn in overeenstemming met de Index Herbariorum, 1, ed. 2, 1954, p. 131–144.

Iedere florist, die meent een bijdrage te kunnen leveren, geschikt voor opname in de Floristische Notities, verzoeken wij deze aan ons in te zenden, geredigeerd in de geest van deze eerste serie.

1. **Tetragonia tetragonoides** (Pall.) O. Ktze.

Noordwijk-binnen, vuilnisbelt, leg. *S. E. de Jongh*, 3 Oct. 1942 (L); Westkapelle, zeestrand, juist boven het hoogste vloedmerk, leg. *A. de Visser*, 20 Aug. 1951 (L); id., leg. *A. de Visser*, 4 Oct. 1951 (L); Noord-Beveland, strand, leg. *A. de Visser*, Oct. 1951 (L); Rotterdam, ruderaal terrein aan de Gustoweg, leg. *S. E. de Jongh, J. H. Kern, S. J. van Ooststroom en Th. J. Reichgelt*, 29 Sept. 1955 (L).

Het tot de *Aizoaceae* behorende geslacht *Tetragonia* L. verschilt van *Mesembryanthemum* L., waarvan één soort, *M. crystallinum* L., hier te lande ook wel eens verwilderd is waargenomen, door het bezit van niet-openspringende vruchten (*Mesembryanthemum* heeft doosvruchten), en door het ontbreken van de bloemkroon.

T. tetragonoides is als volgt gekarakteriseerd: Kruidachtige, vlezige plant. Stengels liggend tot opstijgend. Bladen verspreid, eirond-

driehoekig tot ruitvormig, aan de voet plotseling in de steel versmald. Bloemen okselstandig, meestal alleenstaand, kort gesteeld, klein, geelgroen. Kelkslippen breed, stomp. Bloemkroon ontbrekend. Vrucht hard, droog, ongeveer tolvormig, hoekig, met meestal 4 hoornvormige aanhangsels aan de top.

De soort is afkomstig van de kusten van Australië, Nieuw-Zeeland, Japan, de Pacifische eilanden en Z.-Amerika. Zij wordt bij ons en ook elders als groente (Nieuwzeelandse spinazie) gekweekt, en verwildert op ruderale plaatsen. Opmerkelijk is, dat zij in de laatste jaren hier te lande ook aan de zeekust werd aangetroffen.

2. **Ranunculus ficaria** L. met gevulde bloemen.

Oegstgeest, Hofdijk, leg. *C. J. van Ooststroom*, 16 Maart 1952; herb. *v. O.* no. 15595 (L).

Bloemen met 3 kelkbladen, ca. 30 gele kroonbladen en daarbinnen nog 30 kleine groene blaadjes.

Gevulde bloemen worden volgens Penzig (1) bij deze soort herhaaldelijk in de botanische literatuur vermeld. Een opgave uit Nederland ontbreekt; wel komt in De Levende Natuur 1, 1896, p. 16 een afbeelding voor van een drietal bloemen met een meer dan normaal aantal kelk- en kroonbladen.

1. O. Penzig, Pflanzen-teratologie, ed. 2, 2, 1921, p. 18.

3. **Nasturtium microphyllum** Boenningh. ex Rchb. en **N. officinale** (L.) R. Br. in Nederland (Fig. 1 en 2).

In 1935 publiceerde Manton (14) een artikel over verspreiding, geschiedenis, morphologie en cytologie van de diploide, triploide en wilde tetraploide waterkers; dit werd in 1940 gevolgd door een publicatie van Howard en Manton (7), waarin vermeld wordt, dat de wilde tetraploide plant een allotetraploide bleek te zijn, en in 1946 door een artikel van dezelfde schrijvers (8), waarin naast *N. officinale* (L.) R. Br. een nieuwe soort gepubliceerd werd als *N. uniseriatum* Howard et Manton.

In 1947 verschijnt vervolgens een artikel van Airy Shaw (1), waarin deze aantoont, dat de naam die zeer waarschijnlijk aan *N. uniseriatum* Howard et Manton toekomt, *N. microphyllum* Boenningh. ex Rchb. is. Verder geeft hij enige verspreidingsgegevens van deze soort, en vraagt de aandacht voor een belangrijke publicatie van Irmisch (10), waaruit blijkt dat deze reeds in 1861 de twee onderhavige soorten als var. *brevisiliqua* en *longisiliqua* van *N. fontanum* (Lamk.) Aschrs. uitstekend onderscheiden en scherp gekarakteriseerd heeft. Hierop volgt in 1949 nogmaals een artikel van Airy Shaw (2) over de variabiliteit en de ecologie van de Britse waterkers-soorten, en in 1950 een van Howard en Lyon (5) over de onderscheiding en verspreiding ervan. Eveneens in 1950 verschijnt van de hand van de Belgische botanicus Lawalrée (11) een publicatie over de verspreiding in België. Nog in hetzelfde jaar geeft Hylander (9) een overzicht van de verspreiding van de *Nasturtium*-soorten in Zweden en Denemarken. Hierin betoogt

deze auteur, dat de naam van de tetraploide soort moet zijn *Rorippa microphylla* (Boenningh.) Hyl., en wijst hij op het merkwaardige feit, dat in Denemarken bijna uitsluitend *N. microphyllum* voorkomt, terwijl *N. officinale* er slechts eenmaal is gevonden. In Zweden daarentegen is *N. officinale* veel algemener dan *N. microphyllum*. Hierna volgen nog diverse kortere of langere mededelingen over het voorkomen van de beide *Nasturtium*-soorten in Frankrijk (12), Zwitserland (3) en Duitsland (4, 13).

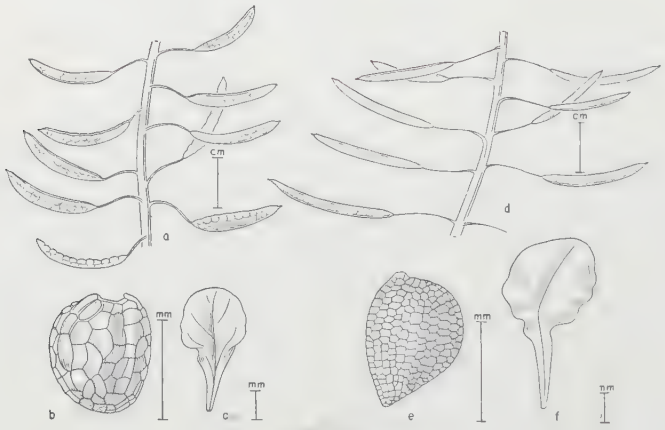

Fig. 1. *a–c*: *Nasturtium officinale* (L.) R.Br., *a*: vruchten, *b*: zaad, *c*: kroonblad; *d–f*: *N. microphyllum* Boenningh. ex Rchb., *d*: vruchten, *e*: zaad, *f*: kroonblad. Naar Ross–Craig (15).

In aansluiting op deze publicaties hebben wij getracht op grond van het materiaal aanwezig in het Rijksherbarium, de herbaria van de K.N.B.V., de Rijksuniversiteit te Utrecht, het Natuurhistorisch Museum te Maastricht en verscheidene particuliere collecties, benevens door veldstudie, ook voor Nederland de verspreiding van de twee soorten vast te stellen. Hierbij bleek, dat *N. microphyllum* door geheel Nederland vrij algemeen voorkomt, doch dat *N. officinale* vrij zeldzaam is en hoofdzakelijk gevonden wordt in Zuid-Limburg, langs de oevers van de grote rivieren en langs de binnenduinrand (zie fig. 2). Wij zagen exemplaren afkomstig van de volgende vindplaatsen: Maastricht, in sloten; tussen Valkenburg en Schin-op-Geul, in sloten, 1895; Epen, 1954; tussen Obbicht en Grevenbicht, Maasoever, 1955; Hedel, oude Maasarm, 1955; Heusden, Doornwaard, oude Maasarm, 1955; Hees bij Nijmegen, aan de Waal; aan de Waal tegenover Rossum; Gorinchem, aan de Merwede, 1911; Werkendam, bij veerdam, 1835; Dordrecht, langs de Merwede, 1914; Numansdorp, 1899; tussen Rotterdam en Kralingse Veer, 1907; Utrecht; Rheden, aan de IJsel; Ellekom, sloot in het IJseldal, 1954; Meppel; tussen Bergen en Egmond, 1927; Haarlem; tussen Leiderdorp en Zoeterwoude, 1899; 's-Gravenhage, Maliebaanspolder, 1844; Renesse, binnenduinrand,

1955. Buiten deze gebieden vallen vondsten van Haren (Gr.) en Appel bij Nijkerk, 1864, waarvan de eerste onzeker is, daar hier zeer goed verwisseling van etiketten kan hebben plaatsgevonden.

Fig. 2. Vondsten van *Nasturtium officinale* (L.) R.Br. in Nederland. Het kruisje geeft een onzekere vindplaats aan.

Deze verspreiding is min of meer in overeenstemming met de opgave van Airy Shaw (2) en van Howard en Lyon (6), dat *N. officinale* in Engeland de neiging vertoont op meer kalkhoudende grond voor te komen.

De beide soorten kunnen op de hieronder aangegeven wijze worden

onderscheiden. Vooral de kenmerken van het zaad zijn zeer duidelijk en met een goede loupe goed waarneembaar.

1. De zaadhuid is bij beide soorten door netvormige lijsten in mazen verdeeld; deze mazen zijn bij *N. officinale* gering in aantal (± 25 op iedere zijde) en groot, bij *N. microphyllum* veel groter in aantal (± 100 op iedere zijde) en klein. Ook bij onrijpe zaden is dit verschil al duidelijk te zien. Fig. 1, b en e.
2. De vruchten van *N. officinale* zijn vrij kort en hebben kortere, dikkere stelen, de zaden zijn duidelijk in 2 rijen gerangschikt. Bij *N. microphyllum* zijn de vruchten en de vruchtstelen langer en slanker, terwijl de zaden (niet altijd even duidelijk) in één rij gerangschikt zijn (naar dit kenmerk hebben Howard en Manton (8) de naam *uniseriatum* aan de soort gegeven). Fig. 1, a en d.
3. De kroonbladen van *N. officinale* zijn ca. 4 mm lang, die van *N. microphyllum* ruim 5 mm. Fig. 1, c en f.
4. De helmknoppen van de lange meeldraden zijn volgens Howard en Lyon (5) bij *N. officinale* intrors, terwijl ze bij *N. microphyllum* extrors zijn.
5. Volgens Rowson in Howard en Manton (8) hebben de twee soorten verschillende stomata-indices. Verdere verschillen waardoor beide in vegetatieve toestand zijn te onderscheiden zijn tot nu toe niet gevonden, hetzij dan, dat gedurende koude perioden in de winter het blad van *N. microphyllum* gemakkelijk paarsbruin kleurt, terwijl dat van *N. officinale* groen blijft.
6. *N. officinale* is diploid (2n = 32), *N. microphyllum* tetraploid (2n = 64). Tussen de twee soorten wordt in de natuur een (triploide) bastaard gevormd, die zich van de ouders onderscheidt door de zeer korte, slecht ontwikkelde vruchten, die bijna geheel steriel zijn. Deze bastaard is tot nu toe nog niet in Nederland gevonden.

1. H. K. Airy Shaw, The botanical name of the wild tetraploid watercress. Kew Bull.1947, no. 1, p. 39–46.
2. H. K. Airy Shaw, Variation and ecology in the British watercress. Rep. Bot. Soc. Brit. Isl. Conf. 1948 (1949), p. 75–76, 3 pl.
3. A. Becherer, Fortschritte in der Systematik und Floristik der Schweizerflora etc., 1950, 1951. Ber. Schweiz. Bot. Ges. 62, 1952, p. 527–582.
4. W. Christiansen, Neue kritische Flora von Schleswig-Holstein, Rendsburg, 1953.
5. H. W. Howard en A. G. Lyon, The identification and distribution of the British Watercress species. Watsonia 1, 1950, p. 228–233, 2 fig.
6. H. W. Howard en A. G. Lyon, Nasturtium R.Br. Journ. Ecol. 40, 1952, p. 228–245, 4 fig.
7. H. W. Howard en I. Manton, Allotetraploid nature of the wild tetraploid watercress. Nature (London) 146, 1940, p. 303–304.
8. H. W. Howard en I. Manton, Autopolyploid and allopolyploid watercress with the description of a new species. Ann. of Bot. New ser. 10, 1946, p. 1–13, 1 pl., 5 fig.
9. N. Hylander, Rorippa microphylla i Sverige och Danmark. Bot. Notiser 1950, p. 1–13, 5 pl., 1 fig.
10. Th. Irmisch, Ueber zwei Varietäten der Brunnenkresse. Bot. Zeit. 19, 1861, p. 316–319.
11. A. Lawalrée, Les Cressons de fontaine. Les Natur. Belg. 31, 1950, p. 28–33, 1 fig.

12. A. Lawalrée, Le Roripa microphylla en France. Bull. Soc. Bot. France 97, 1950, p. 212–213, 1 fig.
13. W. Ludwig, Über einige verkannte Arten der deutschen Flora. Ber. Bayer. Bot. Ges. 30, 1954, p. 84–87.
14. I. Manton, The cytological history of Watercress (Nasturtium officinale R.Br.). Zeitschr. f. ind. Abst. u. Vererbungsl. 69, 1935, p. 132–157, 2 pl., 11 fig.
15. S. Ross-Craig, Drawings of British Plants 3, 1949, pl. 3 en 4.

4. **Silene sericea** All.

Rockanje, opgeslagen in tuin, hoogstwaarschijnlijk toevallig als zaad meegebracht van Corsica, leg. *C. Sipkes*, 1 Sept. 1952 (L).

Herkomst: Balearen, Corsica, Sardinië, Ligurië.
Voor een beschrijving en afbeelding zie Coste (1).

1. H. Coste, Flore descr. et ill. de la France 1, 1901, p. 178.

5. **Prunus spinosa** L. f. **coaetanea** (Wimm. et Grab.) Schneid., Ill. Handb. Laubholzk. 1, 1906, p. 628 — *P. spinosa* L. var. *coaetanea* Wimm. et Grab., Fl. Siles. 1², 1829, p. 10.

Bij deze vorm verschijnen de bloemen tegelijk met de bladen. Zij werd als nieuw voor de Nederlandse flora vermeld in een der lijsten in De Levende Natuur (1). Uit een onderzoek van herbariummateriaal blijkt, dat de vorm al eerder op verscheidene plaatsen werd gevonden en ook reeds werd vermeld door Van Soest (2).

1. A. W. Kloos en S. J. van Ooststroom, Nieuwe plantensoorten en -vormen in Nederland gevonden in 1951. De Lev. Natuur 55, 1952, p. 176.
2. J. L. van Soest, Flora van Arnhem, IV. Ned. Kruidk. Arch. 1925, p. 109.

6a. **Malva sylvestris** L. var. **parvifolia** Schur, Enum. Plant. Transs. 1866, p. 130 (in het register van hetzelfde boek wordt de naam *parviflora* gebruikt!).

Bloemkroon kleiner dan normaal, ca. 12 mm lang.
Deze vorm, die in De Levende Natuur 56, 1953, p. 213 als nieuw voor Nederland werd vermeld, blijkt reeds eerder hier te lande gevonden te zijn door De Wever op enkele plaatsen in Zuid-Limburg; vgl. Kloos (1); ook ligt er in het Rijksherbarium een exemplaar van Rotterdam van deze vorm, leg. *Jansen en Wachter*, Juli 1909.

b. **Malva sylvestris** L. var. **purpurascens** Weston, Bot. Univ. 3, 1772, p. 477 — *M. sylvestris* L. var. *zebrina* W. Mill. in Cycl. Amer. Hort. 2, 1903, p. 970.

Amsterdam, Rietlanden, rangeerterrein, leg. *A. W. Kloos Jr.*, Aug. 1915 (L); Texel, De Cocksdorp, „op verschillende plaatsen langs slooten in bouwland, steeds deze bloemkleur", leg. *A. W. Kloos Jr.*, 21 Aug. 1918 (L); Tilburg, terrein wolwasserij Bern. Pessers, leg. *B. K. Boom, S. E. de Jongh, S. J. van Ooststroom*, 4 Oct. 1952, herb. *v.O.* no. 16636 (L); id., leg. *S. E. de Jongh, S. J. van Ooststroom, Th. J. Reichgelt*, 19 Sept. 1953, herb. *v.O.* no. 17506 (L).

Kroonbladen wit of zeer licht lila, met donker purperen strepen. Zie ook Kloos (1).

1. A. W. Kloos Jr., Aanwinsten van de Nederlandse flora in 1933. Ned. Kruidk. Arch. 44, 1934, p. 123.

7. **Bupleurum semicompositum** L.

Grave, adventief bij een graansilo, leg. *Fr. M. Ludwinus Kleyberg*, Sept. 1952 (L).

Deze soort is na verwant aan *B. tenuissimum* L., doch onderscheidt zich daarvan op de volgende wijze:

B. semicompositum L.	*B. tenuissimum* L.
Omwindsel meestal 4–5–bladig.	Omwindsel meestal 3–bladig.
Omwindseltjes tot 2 maal zo lang als de vruchtschermpjes.	Omwindseltjes even lang als of iets langer dan de vruchtschermpjes.
Vrucht 1–1½ mm lang, met onduidelijke ribben, zwart, met witachtige wratjes.	Vrucht 2–2½ mm lang, met duidelijke, scherpe ribben, bruin, met lichtbruine wratjes.

B. semicompositum komt in verschillende vormen in het gehele Middellandse Zee-gebied voor en werd tot nu toe in Frankrijk, Duitsland en Engeland adventief gevonden.

8. **Angelica sylvestris** L. f. **nidus** (Kittel) Thell. in Hegi, Ill. Fl. Mitt.-Eur. V. 2, 1926, p. 1335.

Woerden, Woerdense Verlaat, leg. *Mevr. J. M. Koops–Bos*, 22 Mei 1952 (L).

Blaadjes van de omwindseltjes breder dan normaal, langer dan de schermpjes en getand. Wel een monstruositeit.

9. **Peucedanum palustre** (L.) Moench f. **involucratum** Cariot et St. Lager, Etudes des fleurs, ed. 8, p. 343, volgens Rouy & Camus, Fl. France 7, 1901, p. 387.

Oukoop, wegrand, leg. *H. Hoogendoorn*, 9 Aug. 1951 (in herb. pr.).

Blaadjes van de omwindsels en omwindseltjes bladachtig. Wel een monstruositeit.

10. **Erica scoparia** L.

Terschelling, heide langs de weg van Oosterend naar het Biologisch Station, 1 exempl., leg. *Th. J. Reichgelt*, 15 Aug. 1952 (L). Later werden in dezelfde omgeving door *K. van Dam*, Dr. *M. F. Mörzer Bruyns* en *H. C. Wesseling* nog een viertal planten aangetroffen.

E. scoparia is van de inlandse *Erica*-soorten direct te onderscheiden door de kleine, tot 2 mm lange, groenachtige bloemkroon. Zij komt voor in het westelijke Middellandse Zee-gebied, van de Kanarische eilanden tot N.W.-Italië en Tunis en reikt in Frankrijk noordelijk tot in de omgeving van Parijs. Zie areaalkaartje bij Irmgard Hansen (1, p. 17).

Oorspronkelijk werd de vondst op Terschelling als zeer merkwaardig beschouwd, daar de afstand tot de tot nu toe bekende meest noordelijke vindplaats ca. 600 km bedraagt en wij hier dus te doen zouden hebben met een opmerkelijke exclave van het areaal van deze zuidelijke soort. Vermoedens over aanvoer tijdens de oorlog door Duitse soldaten en

over een meer natuurlijke verspreiding door trekvogels zijn geuit, doch enige zekerheid daarover is niet te krijgen. Het vermoeden dat wij hier toch met een „oorlogsadventief" te maken hebben wordt echter versterkt door de vondst, in 1955, van *E. ciliaris* op hetzelfde terrein (Zie deze Notities, no. 11). Het is immers zeer onwaarschijnlijk dat twee *Erica*-soorten tegelijkertijd hun areaal zo plotseling naar het Noorden zouden hebben uitgebreid.

1. I. Hansen, Die europäischen Arten der Gattung Erica L. Bot. Jahrb. 75, 1950, p. 1–81.

11. **Erica ciliaris** L.

Terschelling, Oosterend, omgeving van het Biologisch Station, leg. *H. C. Wesseling*, 25 Sept. 1955 (L); oorspronkelijk, in Aug. 1955, daar ter plaatse ontdekt door Dr. Runge, Münster (Westf.).

Deze soort verschilt van *E. tetralix* L., waarmee zij de lang gewimperde bladen en kelkslippen gemeen heeft o.a. op de volgende wijze:

Erica ciliaris L.	*Erica tetralix* L.
Bladen eirond tot langwerpig eirond, in kransen van 3–4.	Bladen kort-lijnvormig, in kransen van 4.
Bloemen in bijna eenzijdige trossen.	Bloemen in schermvormige hoofdjes.
Bloemkroon buis-kroesvormig, 8–10 mm lang, purperrood.	Bloemkroon kroesvormig, 5–7 mm lang, licht- tot donkerrose.
Helmknoppen zonder hoornvormige aanhangsels.	Helmknoppen aan de voet met hoornvormige aanhangsels.
Vrucht kaal.	Vrucht behaard.

E. ciliaris is een streng atlantische soort en komt volgens Irmgard Hansen (lit. zie Fl. Not. no. 10) voor in Marokko, de aan de Atlantische Oceaan grenzende delen van Spanje, Portugal en Frankrijk, verder in Z.W.-Engeland en in W.-Ierland. Evenals bij de vorige soort hebben wij hier waarschijnlijk te doen met een „oorlogsadventief".

12. **Linaria triphylla** (L.) Mill.

Grave, bij een graansilo, leg. *Fr. M. Ludwinus Kleyberg*, 27 Aug. 1952 (L).

Plant eenjarig, kaal, zeegroen. Stengels rechtopstaand, 15–30(–45) cm hoog. Bladen meestal in kransen van 3, soms tegenoverstaand, de bovenste soms verspreid, elliptisch tot omgekeerd eirond, stomp, zittend, 3–5–nervig. Bloemen meestal in een dichte tros. Kelkslippen langwerpig elliptisch, stomp. Bloemkroon (met inbegrip van de spoor) ca. 2 cm lang, witachtig met geel gehemelte en blauwachtige spoor; spoor gekromd, spits, iets korter dan de rest van de bloemkroon. Doosvrucht bijna even lang als de kelk. Zaden driekantig, met grof netvormige lijsten, ongevleugeld.

Adventief uit het Middellandse Zee-gebied.

13. **Centaurium spicatum** (L.) Fritsch.

Leiden, terrein bij de stationswerken, leg. *S. E. de Jongh*, Sept. 1952 (herb. pr.).

In tegenstelling tot de inheemse *Centaurium*-soorten, waarbij de bloemen steeds in duidelijke gevorkte bijschermen gerangschikt zijn, heeft *C. spicatum* bloemen, die schijnbaar in aren staan.

Adventief. De soort komt voor in het Middellandse Zee-gebied, oostelijk tot in Midden-Azië. Aan de Westkust van Frankrijk reikt het areaal volgens Coste noordelijk tot Nantes.

14. **Artemisia stelleriana** Bess.

Oostvoorne, verwilderd in de duinen bij het Biologisch Station „Weevers Duin", leg. *S. J. van Ooststroom* no. 16540, 30 Aug. 1952 (L). Omstreeks 1949 door C. Sipkes daar ter plaatse aangeplant; het oorspronkelijke materiaal was afkomstig uit Portland (Engeland).

Deze soort, die inheems is in Noordoost-Azië werd reeds in de vorige eeuw als sierplant in Noord-Amerika gekweekt en begon daar omstreeks 1880 te verwilderen in de oostelijke Verenigde Staten (2). In Engeland werd zij eveneens gekweekt en verwilderde op enige plaatsen langs de zuidkust; ook bij Dublin (1). Wiinstedt (3) vermeldt gevallen van verwildering in Zweden en Denemarken.

1. A. R. Clapham c.s., Flora of the British Isles, 1952, p. 1086.
2. M. L. Fernald, in Gray's Manual of Botany, ed. 8, 1950, p. 1522.
3. K. Wiinstedt, Nye Bidrag til den danske Flora, no. 29. Bot. Tidsskr. 46, 1943, p. 149.

15. **Leontodon autumnalis** L. f. **concolor** (Körn.).

Twijzel, Fr., Oude Dijk, aan een zandig fietspad, H6.45.34, leg. *D.T.E. van der Ploeg*, 14 Aug. 1952 (herb. pr.).

De randbloemen der hoofdjes vertonen niet als gewoonlijk aan de onderzijde een rode streep, doch zijn geheel geel.

16. **Schkuhria pinnata** (Lamk.) O. Ktze. in Nederland (Fig. 3).

Kloos (3, 4, 5) vond in 1913 te Wormerveer een vijftal exemplaren van een hem onbekende adventieve Composiet. Thellung, aan wie enige der planten werden toegestuurd, determineerde ze ten dele als *Schkuhria advena* Thell., ten dele als *S. pinnata* (Lamk.) O. Ktze. Volgens Cabrera (1) en Heiser (2) moet echter *S. advena* Thell. als een jonger synoniem van *S. pinnata* (Lamk.) O. Ktze. worden beschouwd, terwijl de door Thellung als *S. pinnata* opgevatte plant door Cabrera als var. *abrotanoides* (Roth) Cabrera tot deze soort wordt gebracht.

S. pinnata var. *pinnata* en var. *abrotanoides* onderscheiden zich alleen door de vorm van de 8 pappusschubben. Deze zijn bij var. *pinnata* alle ongenaald en meer of minder stomp (fig. 3,a) terwijl er bij var. *abrotanoides* 4 ongenaald zijn en 4 genaald (fig. 3,b).

Van var. *pinnata* zagen wij in het Rijksherbarium en het herbarium van de K.N.B.V. de volgende exemplaren:

Wormerveer, bij meel- en oliefabrieken, *Kloos*, Sept. 1913; id., bij pakhuis, *Kloos*, Aug. 1917; Rotterdam, rangeerterrein, *Kloos*, Oct. 1917; 's-Hertogenbosch, De

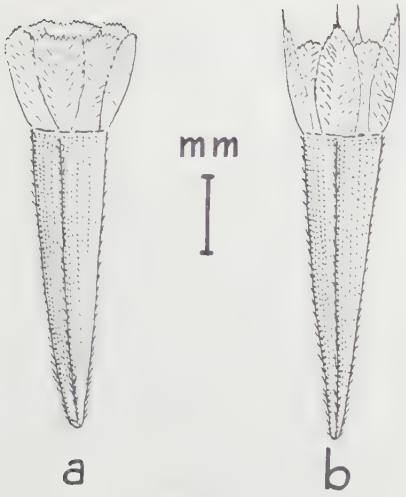

Fig. 3.
a: vrucht van *Schkuhria pinnata* (Lamk.) O.Ktze, var. *pinnata*; *b*: vrucht van *id.*, var. *abrotanoides* (Roth) Cabrera.

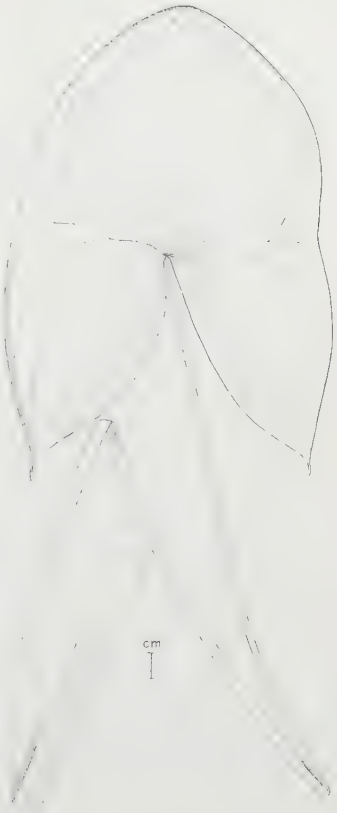

Fig. 4.
Blad van *Sagittaria latifolia* Willd. var. *obtusa* (Mühlenb. ex Willd.) Wieg.

Pettelaar, *Van Giersbergen*, Juli 1937; Tilburg, bij een wolfabriek, *Kern en Reichgelt*. Aug. en Oct. 1939; id., *Kloos*, Oct. 1951; id., *Van Ooststroom en Reichgelt*, Oct. 1954, Voorts: Rotterdam, *de Jongh*, 1948 (in herb. pr.).

Van var. *abrotanoides* (Roth) Cabrera:

Deventer, bij meelfabriek, *Henrard*, Aug. 1917; id., Pothoofd, *Reuvekamp*, Oct. 1948; Wormerveer, bij meelfabriek, *Kloos*, Aug. en Sept. 1913, Aug. 1914; Rotterdam; graanadventief, *Jansen en Wachter*, Sept. en Oct. 1920; Tilburg, bij wolfabriek, *Pater Ludovicus*, Oct. 1938; id., *Kloos*, Aug. 1940; id., *Kloos*, Oct. 1947; id., *Kloos*, Aug. 1950; id., *Van Ooststroom*, Oct. 1950; id., *Kloos*, Sept. 1951; id., bij wolwasserij, *Kloos*, Oct. 1947.

1. A. L. Cabrera, Notas sobre Compuestas de la República Argentina II. Anal. Soc. Cient. Argent. 114, 1932, p. 182–195.
2. Ch. B. Heiser Jr., A revision of the genus Schkuhria. Ann. Missouri Bot. Gard. 32, 1945, p. 265–278.
3. A. W. Kloos Jr., Ned. Kruidk. Arch. 1913, p. 60.
4. A. W. Kloos Jr., Aanwinsten van de Nederlandsche flora in 1914. Ned. Kruidk. Arch. 1914, p. 73.
5. A. W. Kloos Jr., Enkele aangevoerde Composieten I. De Levende Natuur 22, Nov. 1917, p. 246–249, 2 fig.

17. **Sagittaria latifolia** Willd. (Fig. 4).

Brummen, „in een sloot, op enige afstand van een oude buitenplaats in het gehucht Leuvenheim; daar ter plaatse al 3 jaar achtereen in bloei waargenomen," leg. *W. van Zwol*, 12 Sept. 1951 (L); id., 17 Oct. 1953 (L).

Deze soort verschilt van het Europese materiaal van *S. sagittifolia* L. in hoofdzaak op de volgende wijze:

S. latifolia Willd.	*S. sagittifolia* L.
Twee- of eenhuizig.	Eenhuizig.
Kroonbladen geheel wit, tot ruim 2 cm lang.	Kroonbladen wit met purperen nagel, tot ca. 1,5 cm lang.
Helmknoppen 1,5–2 mm lang, geel tot geelbruin.	Helmknoppen ca. 1 mm lang, bruinrood tot purper.
Nootjes in omtrek omgekeerd eirond, met lange, laterale, vrijwel horizontale snavel.	Nootjes in omtrek omgekeerd eirond of bijna cirkelrond, met korte, apicale, rechtopstaande snavel.

Daar de planten van Brummen mannelijk zijn, is de beschrijving der nootjes gebaseerd op buitenlands materiaal.

S. latifolia is een Amerikaanse soort, die volgens Bogin (3) voorkomt van Canada tot het N.W.-deel van Zuid-Amerika. In W.-Indië is zij volgens deze auteur waarschijnlijk ingevoerd, evenals in Hawaii. Bij ons wordt of werd de soort wel als sierplant gekweekt. Bergmans (1) vermeldt haar als zodanig; Boom (4) echter niet. Het is waarschijnlijk dat de vondst bij Brummen op verwildering betrekking heeft; volgens de opgave van de heer Van Zwol blijkt de soort op genoemde vindplaats stand te houden en zich zelfs uit te breiden. In 1951 kwam zij, naar de vinder ons mededeelde, in de sloot al over een lengte van ca. 30 m voor.

In Europa is de soort reeds op enige plaatsen aangetroffen en zelfs hier en daar ingeburgerd. Er zijn opgaven bekend van Frankrijk (5), Zwitserland (7), Duitsland (2, 7), Italië (8) en Bulgarije (6).

S. latifolia is zeer variabel in bladvorm; de vorm van de eindlob der pijlvormige bladen kan variëren van lijnvormig tot eirond-driehoekig evenals die van de basale lobben. Onze plant heeft brede, eirond driehoekige, stompe bladen en behoort tot de var. *obtusa* (Mühlenb. ex Willd.) Wieg. (fig. 4).

Een plant, gevonden door Dr. A. J. Ultee in een duinpannetje op de Waalsdorpervlakte bij 's-Gravenhage, 7 Sept. 1954 (L), behoort zeer waarschijnlijk ook tot deze soort; het materiaal dat wij ontvingen is echter te fragmentarisch voor een volkomen zekere determinatie.

1. J. Bergmans, Vaste planten en rotsheesters, 1924, p. 479.
2. A. Binz, Ergänzungen zur Flora von Basel. Verh. Naturf. Ges. Basel 62, 1951, p. 248–266.
3. C. Bogin, Revision of the genus Sagittaria (Alismataceae). Mem. New York Bot. Gard. 9, 1955, p. 179–233, 20 fig.
4. B. K. Boom, Flora der gekweekte kruidachtige gewassen 2, 1950.
5. H. Glück, in Pascher, Süsswasserfl. Mitt.-Eur. 15, 1936, p. 106.
6. D. Jordanoff, Neue und seltene für Bulgarien Pflanzen (sic!). Bull. Soc. Bot. Bulg. 5, 1932, p. 59–62.

7. H. Stauffer, Sagittaria latifolia Willd. in der Schweiz. Ber. Schweiz. Bot. Ges. 64, 1954, p. 135–138, 2 fig.
8. C. Stucchi, Sagittaria latifolia L. (sic!) nel Varesotto. N. Giorn. Bot. Ital. N.S. 57, 1950, p. 272–273.

18. **Verwilderde sierplanten.**

Wij vermelden deze, omdat bij het verwilderen van sierplanten vaak een kans op inburgeren bestaat en het ons daarom nuttig voorkomt de vondsten te signaleren.

a. **Cleome spinosa** Jacq.

Winterswijk, op oude vuilnisbelt, leg. *J. H. Schouten*, 10 Sept. 1951 (L).

Herkomst: Oorspronkelijk uit Midden- en tropisch Zuid-Amerika; adventief in de tropen van de Oude Wereld.

b. **Apios americana** Med. (*A. tuberosa* Moench).

Nieuwendam (N.H.), spontaan in tuin, leg. *J. Visser Szn.*, 17 Sept. 1951 (L).

Herkomst: Oostelijke Verenigde Staten.

c. **Indigofera gerardiana** Baker.

Aan de straatweg Dordrecht-Moerdijk, tussen puin, leg. *A. G. de Wilde* no. 4517, 12 Aug. 1951 (L en herb. pr.).

Herkomst: Himalaya.

d. **Cornus alba** L.

Leiden, ruderaal terrein, leg. *S. J. van Ooststroom* no. 9932, 24 Mei 1948 (L); Sinderen bij Varsseveld, verwilderd langs wegrand, leg. *S. J. van Ooststroom* no. 16503, 8 Aug. 1952 (L); tussen Koten en Lutkepost (gem. Achtkarspelen), leg. *M. T. Jansen en D. T. E. van der Ploeg*, 1953 (herb. Van der Ploeg).

Herkomst: Noord- en Noordoost-Azië.

e. **Ageratum houstonianum** Mill. (*A. mexicanum* Sims).

Heelsum, stortterrein, leg. *S. E. de Jongh*, Aug. 1938 (L); Leiden, spontaan in tuin, leg. *L. D. Brongersma*, 23 Aug. 1943 (L); id., wegkant, leg. *R. C. Bakhuizen van den Brink fil.* no. 5604, 28 Oct. 1944 (L); Dordrecht, ruderaal terrein bij de werf „De Biesbos", leg. *A. G. de Wilde* no. 5559, 12 Oct. 1952 (L en herb. pr.).

Herkomst: Oorspronkelijk uit Midden- en Zuid-Amerika; thans in de tropen wijd verspreid.

f. **Heliopsis helianthoides** (L.) Sweet var. **scabra** (Dun.) Fern. (*H. scabra* Dun.).

Leiden, spontaan in tuin, leg. *S. E. de Jongh*, 12 Nov. 1952 (L); Haren (Gr.), ruderaal terrein aan de Osdijk in de Oostpolder, leg. *Unio*, 21 Juli 1953 (L).

Herkomst: Noord-Amerika.

g. **Allium christophi** Trautv. (*A. albopilosum* C. H. Wright).

Provinciale duinen bij Castricum, in een duinpan, leg. *J. Bansberg*, Sept. 1951 (L).

De heer Bansberg vond op genoemde plaats *11n* exemplaar. Het is vermoedelijk als een verwilderde sierplant te beschouwen; de mogelijkheid van opslag uit fazantenvoer is echter niet uitgesloten. De soort komt volgens Vvedensky (2) voor in W.-Turkestan. Voor een

beschrijving moge verwezen worden naar deze auteur en naar Boom (1, als *A. albopilosum*).

1. B. K. Boom, Flora der gekweekte kruidachtige gewassen 2, 1950, p. 346.
2. A. J. Vvedensky, The genus Allium in the U.S.S.R., transl. by H. K. Airy Shaw. Herbertia, 11, 1944 (1946) p. 201.

SUMMARY

These "Floristische Notities" contain notes on the phanerogamic flora of the Netherlands. In this first series are mentioned:

1. *Tetragonia tetragonoides* (Pall.) O.Ktze., found in waste places, on rubbish-heaps, and also on the beach in a few localities in the province of Zeeland.
2. A specimen of *Ranunculus ficaria* L. with flowers composed of 3 sepals, c. 30 yellow petals and instead of the stamens and pistils c. 30 minute green leaves.
3. *Nasturtium microphyllum* Boenningh. ex Rchb. and *N. officinale* (L.) R.Br. Differences between these two species are given (see fig. 1) and the distribution in the Netherlands of the latter is discussed (see map, fig. 2).
4. *Silene sericea* All. Most probably casually introduced from Corsica.
5. *Prunus spinosa* L. f. *coaetanea* (Wimm. et Grab.) Schneid. Found in several localities in the Netherlands.
6. *Malva sylvestris* L. var. *parvifolia* Schur and var. *purpurascens* Weston. Both varieties introduced and probably also native.

7 and 12. *Bupleurum semicompositum* L. and *Linaria triphylla* (L.) Mill. Introduced near a granary at Grave (prov. of N. Brabant).

8 and 9. *Angelica sylvestris* L. f. *nidus* (Kittel) Thell. and *Peucedanum palustre* (L.) Moench f. *involucratum* Cariot et St. Lager. New records for the Netherlands.

10 and 11. *Erica scoparia* L. and *E. ciliaris* L. Both species probably unintentionally introduced during World-War II in the island of Terschelling, and at least the former becoming naturalized there.

13. *Centaurium spicatum* (L.) Fritsch. Found as an alien near the railway station at Leyden.
14. *Artemisia stelleriana* Bess. Cultivated and run wild in the dunes of the island of Voorne.
15. *Leontodon autumnalis* L. f. *concolor* (Körn.). New record for the Netherlands.
16. *Schkuhria pinnata* (Lamk.) O. Ktze., var. *pinnata* and var. *abrotanoides* (Roth) Cabrera. Differences between these two aliens (see fig. 3), and a list of localities are given.
17. *Sagittaria latifolia* Willd. Naturalized in at least one locality (a ditch near Brummen, prov. of Gelderland); also found near The Hague in a wet, shallow dune valley (fig. 4).
18. A list of ornamental plants, escaped from cultivation, of which *Cornus alba* L. is naturalized in a few localities.

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THE INFLUENCE OF LIGHT ON THE LOSS OF LABELLED PHOSPHORUS FROM BEAN LEAVES

BY

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(*received January 27th, 1956*)

Introduction

One advantage of using labelled substances is that with the aid of the appropriate apparatus the course of uptake and loss by plant organs can easily be traced with a single intact plant. However, in the field of plant physiology only a few such *in vivo* experiments have been performed.

In 1940 Brewer and Bramley reported some results on P and Na uptake with maize plants. They studied the influence of light on uptake and loss by leaves and found striking results.

The influence of light on loss of substances from leaves is of particular interest in relation to the transport mechanism. It has been proved that the downward transport of 2,4-D from bean leaves only occurs if there is a simultaneous transport of sugar (Weintraub and Brown 1950, Mitchell 1951). This fact is considered to be a proof for the presence of a mass flow as is assumed in Münch's theory on transport in sieve tubes.

Therefore, in the experiments reported here, the influence of light on the loss of labelled phosphorus, previously accumulated in leaves of bean plants, was studied.

Using Martin's continuous recorder (Martin 1952) in the Department of Agriculture, Oxford, experiments were started by the first author at the instigation of Dr. R. Scott Russell. Significant though slight effects, owing to the short experimental periods, were obtained. These investigations were continued in the Botanical Laboratory, Groningen, using longer experimental periods. Here only the results of these long-term experiments will be briefly discussed. It is realised that a satisfactory analysis will not be possible until much more work has been done.

Material and methods

Intact bean plants (*Phaseolus vulgaris*), grown on a complete aerated Hoagland solution were used at an early stage of development, the primary leaves being fully expanded, while the first trifoliate leaf showed incipient unfolding.

The pretreatment was given in a constant-temperature room (20° C), in which also the experiments were performed. Fluorescent light was used for illumination.

Individual bean plants were placed in a Hoagland solution with labelled phosphorus (15 mg P/liter, 15–25 micro Curie P^{32}/liter), the period of absorption being dependent on the rate of absorption and accumulation in the leaf. The figures show that the final activity varied greatly between experiments. Redistribution was then studied while the plant was in a minus-P solution.

The course of the labelled phosphorus content of one of the opposite leaves was traced by placing a counter tube on the underside of the leaf. Philips counting equipment PW 4020, GM 4810 with counter tube 18513 (effective area 0.3 cm^2) was used.

Experiments

Figure 1A shows the results obtained with a young bean plant, which was allowed to absorb labelled phosphorus from a Hoagland solution for a 10 hours period. It was then placed on a Hoagland minus phosphorus solution for the rest of the experiment. The increase of the activity of one of the opposite leaves was studied. This leaf was kept in the light during the first 5–6 days, the other aerial parts being kept in the dark as schematically indicated at the top of the figure. The activity continued to increase for another three days, although the roots were placed in a minus-P solution. A maximum value was reached on the third day after which a rapid fall of the labelled phosphorus content could be observed. This fall continued to occur during the first two days in the following period, in which the whole plant was in the dark. But it was gradually changed into an increase. When, on the 7th day, the lowest value was reached, the total loss amounted to 10–12 % of the maximum value obtained on the third day of the experiment.

The leaf being placed once more in continuous light, a slight decrease could be observed during the first few days, but then hardly any further decrease occurred. This apparent inability to show any back transport whatsoever, was always observed at the end of these long-term experiments. The slow deterioration of the leaves is believed to explain this effect. This stresses the fact, that the losses observed depended on the metabolic activity of healthy leaves.

Figure 1B shows the results of a similar experiment in which the whole plant was in continuous light, except for one of the opposite leaves, the phosphorus content of which was traced, and which was kept alternately in the light and in the dark for some days as indicated in the figure. Absorption was slow; consequently the absorption period was extended to 3 days. A maximum value for the labelled phosphorus content was then reached. During the redistribution period the plant was on a minus-P solution. The pattern of changes was in accordance with that of the previous experiment.

Fig. 2 shows the results of an experiment in which the treatment

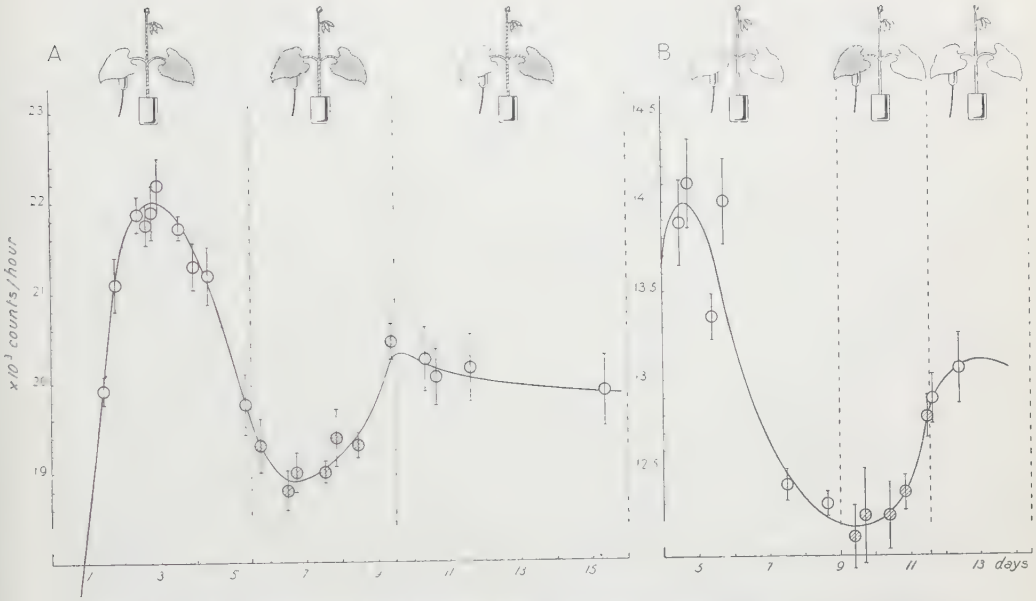

Fig. 1. The influence of light and dark on the redistribution of labelled phosphorus from a bean leaf. The bean plant was allowed to absorb a definite amount of labelled phosphorus from a Hoagland solution. Redistribution took place while the plant was in a phosphorus-free nutrient solution. During the redistribution period the plants were kept either in continuous light (B) or in continuous dark (A) exept for one of the primary leaves which was placed alternately in the light and the dark as shown at the top of the figure.

The labelled phosphorus content of the experimental leaf was determined by means of a Geiger-Müller tube, directly applied to the leaf, and is expressed in activity units. No importance should be attached to the differences in activity level between various experiments as they are mainly due to a variety of irrelevant factors like specific activity of the solution, position of the counting tube, the length of the absorption period etc. Finally, it should be realized that the fluctuations in labelled phosphorus content are small compared with the total amount of labelled phosphorus present in the leaf.

applied to the plant was in a way the reverse of the treatments in the previous ones.

The plant used was at a somewhat older stage; the first trifoliate leaf was very well developed, the size of the second trifoliate leaf being about 34 mm. The two opposite leaves were in a perfect condition and it was possible to extend the duration of the experiment, which lasted for almost 4 weeks.

This plant was allowed to absorb labelled P from a Hoagland solution for 47 hours, the whole plant being kept in the light. At the end of this period the plant was again transferred to a minus-P solution. During this period as well as during the following redistribution period the activity of one of the opposite leaves was studied. This leaf remained in the light, the other parts of the shoot being kept either in the light or in the dark. The labelled P content continued to increase for a while after the plant was put into minus-P

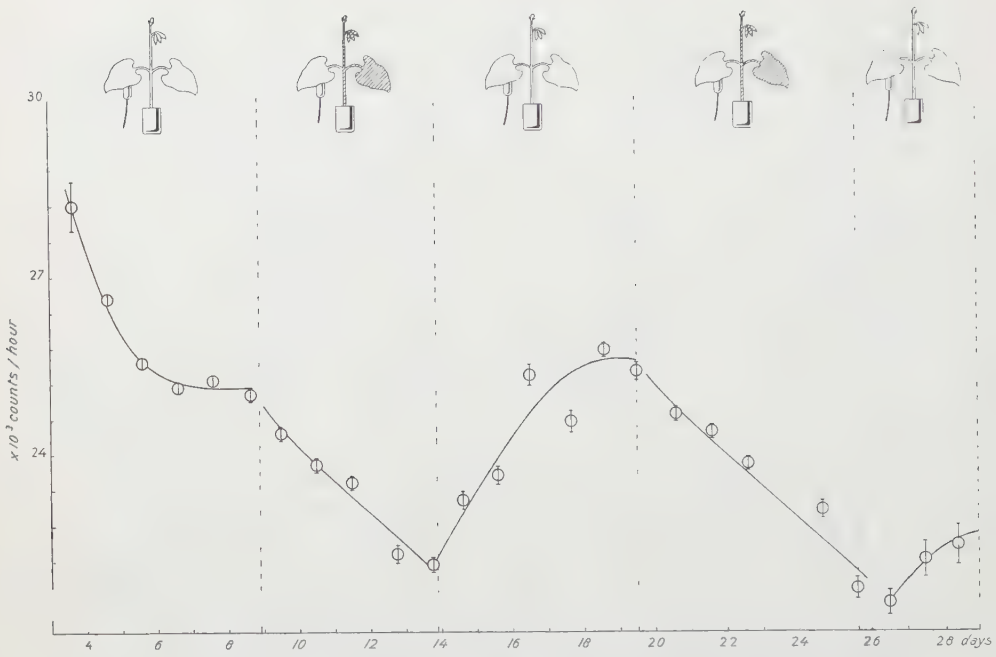

Fig. 2. The influence of the light conditions on the redistribution of labelled phosphorus from a bean leaf. During the redistribution period the plant was in a phosphorus-free nutrient solution. The light-dark treatments are schematically shown at the top of the figure: the experimental leaf was in continuous light, the other parts being either in the light or in the dark.

solution. A maximum value was soon reached and then a gradual decrease could be observed until after 5–6 days a more or less steady state was obtained. Then the greater part of the plant was placed in the dark. A further decline was induced which continued until the end of this dark period. But as soon as the whole plant was replaced in the light this fall was changed into an equally rapid increase of the labelled P content. After 4–6 days about the same activity-level was reached as at the end of the first light period. The perfect condition of this plant made it possible to repeat this dark and light treatment, and to confirm the results so far obtained, although during this second part of the experiment the trifoliate leaves showed an incipient colour changing so that, finally, the experiment had to be cut short.

Discussion

The loss of labelled phosphorus from a leaf, pretreated in such a way that its initial phosphorus content was high, was found to be promoted by illumination of this leaf. However, such a loss could only be observed a few days after the plant was transferred from a labelled nutrient solution to a minus-phosphorus solution. Initially the increase in labelled phosphorus content continued for a few days until a maximum was reached. This increase must be due to a continuation of the

supply from the roots. The fact that this initial increase in labelled phosphorus content was gradually changed into a fall suggests that the actual labelled phosphorus content of the leaf is at any time the result of an influx into and an efflux from the leaf. At any rate, in the opinion of the present authors the effects obtained in these experiments by the various light and dark treatments, can most readily be explained by assuming a continuous circulation of phosphorus within the plant (Arisz 1953, Biddulph 1941, Mason and Maskell 1931). It is very unlikely that in the dark the transpiration stream carrying labelled phosphorus from the roots to the leaf is increased. Nor is an increased fixation of phosphorus in the leaf very likely. On the contrary, both processes will rather tend to decrease the phosphorus content if the leaf is kept in the dark. When in spite of this, a gain in phosphorus was observed this must be due to a retarding effect of the dark treatment on the efflux processes.

Weintraub and Brown (1950) demonstrated that the transport of 2,4-D was connected with the transport of sugars from the leaf to which the growth substance was applied. The same may very well apply to the effect observed on the transport of phosphorus. It is agreed that if sugars would be transported as phosphorylated sugars, as they are often assumed to be, this conclusion would be almost self-evident. However, according to Wanner (1952) carbohydrates in the sieve tubes occur only in their simplest forms owing to the presence of large amounts of phosphatases. Moreover, the preliminary results from experiments, now in progress in this laboratory, show similar effects for labelled rubidium. This suggests the presence of a mass flow from the leaf as assumed in Münch's theory, effected by the sugar production of the leaf.

So far only the influence of light on the leaf in which the activity was measured has been considered. The condition of the other parts of the plant appeared to be of equal importance. Good results could only be obtained if rapidly growing parts were present, which apparently constituted a sink (Williams 1954). Moreover, changing the light conditions of these parts of the shoots proved to have a profound influence on the labelled phosphorus status of the experimental leaf. Continuing the previous line of thought the lowering of the phosphorus level in this leaf, if the other parts are kept in the dark, may be explained by assuming a lower efflux from these parts, being in the dark, to the experimental leaf. A smaller supply of phosphorus from the other parts is conceivable as the loss of phosphorus from the root tissue to the xylem vessels will depend on the supply from the shoot and the general metabolic activity, both of which are reduced in the dark. In addition there may be an enhanced loss from the leaf as all other parts, in particular the young ones, become dependent on the sugar supply from this leaf.

Thus starting from the idea of a circulation of phosphorus within the plant and the promoting effect of sugar from photosynthesis on the transport through the sieve tubes, a satisfying explanation for the effects observed can be given.

So far experimental data have been discussed as if they represent total phosphorus contents rather then labelled phosphorus contents of the leaf. In fact, alterations in activity can also be explained by assuming changes in specific activity, without any net gain or loss of phosphorus. Therefore the course of the specific activity was studied with a group of bean plants, treated in a way similar to that in the experiments discussed. The results were equal to the expectations. It was found, that at the end of the absorption period specific activity was highest in the roots. During the redistribution period the specific activity of the root phosphorus decreased gradually, while the specific activity of all other parts of the plant increased until after about 5 7 days the specific activity was almost the same for any part of the plant. In particular, no decrease of specific activity in any part of the shoot could be observed. This means that the decreases in activity observed in the experiments described above represent real net losses from the leaf. As a matter of fact, in some cases the net loss may have been a little higher.

On the other hand, the increases in activity, must have partly been due to an increase of the specific activity at least during the first few days of the experiment. However, the results represented by figure 2 show that the changes in specific activity were of minor importance for the greater part of the experiment. Otherwise it would not have been possible to obtain such strikingly similar results in the subsequent light-dark treatments.

Finally it should be realised, that also in case of a mere exchange, i.e. a change in total activity without any change in total phosphorus content, a simultaneous loss and gain of phosphorus must be assumed to occur. Several mechanisms are conceivable but, no doubt, the most rapid one is by way of a supply through the xylem vessels and a loss through the sieve tubes.

SUMMARY

Young bean plants were allowed to absorb a limited amount of labelled phosphorus and were then placed in a minus phosphorus solution. The labelled phosphorus content of one of the primary leaves was studied by a Geiger-Müller tube directly against the leaf. During the first few days of the experiment the labelled phosphorus content increased gradually up to a maximum value. It was followed by a decrease until a constant level was reached, provided the period was long enough. This decrease of the labelled phosphorus content could be stopped and even changed into a increase simply by placing the test leaf in the dark. These effects were obtained irrespective of the light conditions of the other parts of the plant.

However, these conditions did affect the phosphorus status of the leaf. A further loss could be induced by darkening the other parts of the plant. Subsequent illumination of these parts caused the labelled phosphorus content in the experimental leaf to increase again. These results were thought to be consistent with the hypothesis of a continuous circulation of the phosphorus in the plant, involving a steady migration of labelled phosphorus into and out of the leaf, the latter being coupled to the stream of assimilates which is influenced by the light conditions of the leaf.

The question of the role played by changes in specific activity was briefly entered into.

ACKNOWLEDGEMENTS

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The first author is much indebted to Dr. R. Scott Russell and Dr. R. P. Martin for their stimulating advise and to the other members of the staff of the Isotope Section of the Department of Agriculture for their kind help during his stay in Oxford.

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THE CONCEPTS ON WHICH A MORPHOLOGY OF THE VASCULAR PLANTS SHOULD BE BASED

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It can hardly be denied that the expression "General Plant Morphology", which is so often met with in botanical textbooks, has little or no meaning. A general morphology of the Plant Kingdom would have to occupy itself with those morphological features that are common to all groups of plants, which means that it would have to confine itself to the common features of the cell structure and eventually to such peculiarities as are independent of the uni- or pluricellular structure of the plant body, e.g. its enclosure within a rigid envelop. However, when we realize that there is in this respect no fundamental difference between the common features of plants and animals or, at least, of some groups of animals [1]) it will be clear that the use of the expression "General Plant Morphology" is misleading and should be avoided.

What in most botanical textbooks is understood by "General Morphology" is not a morphology of the whole Plant Kingdom but only of a part of it; however, the delimitation of this part, and this is a most astounding feature, is but seldom explicitly indicated, and, moreover, proves to vary, sometimes even in different chapters of the same work. Most textbook-writers seem to agree that *Algae* and *Fungi* have a morphology of their own, and that the latter should be left to specialists in these fields; they accordingly restrict their attention either to the *Embryophyta*, i.e. the group which comprises the *Bryophyta* and the Vascular Plants, or to the Vascular Plants alone.

Morphology, of course, should deal with a well-defined group, for its object is the investigation of the plan according to which the body of the representatives of the group that has been chosen is constructed, and only in a well-defined group the presence of such a plan is to be

[1]) This apparently means that the generally accepted division of the organisms in animals and plants is unacceptable, for it is certainly impossible that groups for which no definite set of general differences can be indicated, should be regarded as natural ones, and as it also seems impossible to change their delimitation in such a way that this end could be achieved, the division into two main groups is definitely to be rejected. An acceptable division can be obtained only by recognizing a larger number of main groups.

expected. Now Engler's group *Embryophyta* is undoubtedly a natural, i.e. a well-defined, unit, as the plants that are brought together under this heading appear to agree in important points, viz. in the antithetic alternation of a haploid and a diploid generation, and especially in the structure of the reproductive parts of gametophyte as well as of sporophyte, the common feature being the cellular structure of the wall by which the mass of reproductive cells is surrounded; only in cases of extreme reduction, viz. when the male gametophyte assumes the character of a pollen tube, these cells are wanting. A general morphology of this group of plants would have to confine itself to these features with, perhaps, an excursion into the domain of plant anatomy in order to pay some attention to the less exceptional but still rather characteristic structure of the growing points.

Of the two groups that have been brought together in the *Embryophyta*, that of the Vascular Plants is probably the best-defined one. It is well characterized by the strongly pronounced dominance of the sporophyte over the gametophyte, and by the peculiar internal differentiation that is met with in the vegetative part of the sporophyte. The points of resemblance in the outward form are, as will be discussed further on, of a more or less controversial nature, and are here for this reason omitted. The *Bryophyta* do not impress us in the same measure as a natural unit, for apart from the dependence of the sporophyte upon the gametophyte, the groups that have been brought together under this name, seem to have little in common. The absence of the internal differentiation found in the Vascular Plants is a purely negative character, and therefore of no account, and in the absence of common characters of a more positive nature the possibility that the dependency of the sporophyte on the gametophyte might have developed independently in the various groups, or in some of them, should receive careful consideration. However, as I do not intend to deal with the morphology of these groups, the question whether they are all correctly included in the *Bryophyta* or whether some of them would deserve a more independent position, may here be left out of consideration.

The textbooks that in the part dealing with morphology restrict their attention to the Vascular Plants, pay, as a rule, but little attention to the common features of this group. They base their treatment mostly on the rich material that former generations of botanists have brought together in the study of its most important subdivision, viz. that of the Angiosperms. Historically this is, of course, easily comprehensible, as Linné, Goethe and A.P. de Candolle, the men by whom the foundations of this morphology were laid, were but superficially acquainted with the other groups of Vascular Plants, and as the morphologists of the next period were more attracted by the great diversity displayed by the Angiosperms and by some other groups of Phanerogams than by the comparative uniformity that prevails in the remaining Vascular Plants. From a scientific point of view the neglect of these other groups is to be regarded as most regrettable, for it was not realized in good time that the base on which classical morphology

was built, was too narrow, and that concepts that were developed in the study of this limited domain, and that in the latter had proved most valuable, were not necessarily applicable in the wider field, where, indeed, their introduction has led to much confusion.

However, before entering into the problems that offer themselves to us in the wider field of the Vascular Plants, we will do well to consider the common features of the latter in somewhat greater detail.

In the structure of the gametophyte there are in the Vascular Plants apparently no common features that are not found also among the groups that have been brought together in the *Bryophyta*, and the gametophyte therefore needs no further consideration.

In the highly developed sporophyte, on the other hand, rather striking common characters are met with, of which, as stated above, no equivalent is found in the Bryophytes. This does not apply to the reproductive parts, for here, as in the gametophyte, no common characters seem to be present that are not duplicated in the *Bryophyta*. In the vegetative part, however, the situation is quite different. Here we find in the first place in the internal structure a differentiation of an epidermis provided with stomata of a quite distinct pattern, and of a stele consisting of xylem and phloem elements. Then there is also a differentiation of the outward form, but here the interpretation of the parts to which this differentiation has led, offers, as I hinted at above, considerable difficulties, and what in reality are merely analogous developments have often uncritically been accepted as homologies. However, with regard to one particularity of the outward form, viz. the presence of roots, there can hardly be difference of opinion. Their endogenous origin as well as the presence of the calyptra appear to be sufficient proof of their homology. The morphological identity of the leaves that we observe in the different groups, on the other hand, may seriously be questioned, and if the leaves should prove to be of different morphological value, it can hardly be doubted that the stems of the different groups too will have to be interpreted as analogous structures.

In order to solve this problem in the right way we should attack it by means of the principle on which all morphological conclusions are to be based. This is that all parts of the same rank occupy a definite position with regard to each other, and that deviations from this position are always but spurious and to be explained by means of a few auxiliary hypotheses, of which classical morphology recognized three, viz. abortion, splitting and concrescence. The first two can still be accepted in the original form, but instead of concrescence it seems preferable to introduce the expression "intercalary growth", which describes what is actually observed in cases that so far have been explained by the aid of concrescence, and seems therefore more suitable. This, however, is here a point of minor importance.

The questions we have to solve are therefore 1^0 what are the main parts of the sporophyte, and 2^0 what position occupy these parts with regard to each other.

The first question is easily answered. In the sporophyte we find just as in the gametophyte a juxtaposition of a vegetative part and a repro-

ductive part. That the presence of the latter, in the sporophyte as as in the gametophyte, is a necessary condition, is obvious, for without the reduction division in the spore-mothercells formed in the reproductive part of the sporophyte, and without the fusion of the sexual cells originating in the reproductive parts of the gametophyte, the antithetic alternation of generations could not be maintained. It is true that the original reproductive part of the sporophyte is occasionally replaced by one of different morphological value, viz. by one that is derived from the vegetative part (vegetative reproduction), but as this is obviously a side-issue, it can not obscure the significance of the original reproductive part. That the vegetative part too is to be regarded in the Vascular Plants as an essential component of the sporophyte, need not be doubted either; the reproductive part is for its food supply entirely dependent on it. However, even in the *Bryophyta*, where the sporophyte lives parasitically on the gametophyte, and where the vegetative part therefore is not needed for the food supply of the reproductive part, it is never entirely suppressed.

The question what position the two main parts of the sporophyte, viz. the reproductive part and the vegetative part, occupy with regard to each other, is not so easily answered. Actually there is a considerable degree of diversity in the local relation between the two parts in the various groups of Vascular Plants. In the first place there is a great variability in the number of the reproductive parts. Sometimes there are but a few of them, but as a rule they are very numerous, and then they are either more or less evenly scattered over definite portions of the vegetative part, or they are arranged in groups that are more or less evenly scattered. When their number is small, the vegetative part proves to be divided into more or less equivalent branches, which bear the reproductive parts at their top, but such a terminal position of the reproductive parts is never met with in the groups where their number is very large. Now, from the standpoint of idealistic morphology it is irrelevant what arrangement we choose as our norm, but once we have made our choice, we will have to show that the other modes of arrangement can be derived from this norm by the aid of one or more of the auxiliary hypotheses that we have mentioned above, and, if necessary, of one or more additional ones.

The two norms that seem to deserve special consideration, are 1⁰ the sparsely branched sporophyte whose subequal ramifications end in a single reproductive part, the situation that is met with in the *Psilophytopsida* (*Psilophytales*), and 2⁰ the sporophyte with numerous reproductive parts occupying lateral positions on the vegetative part or eventually on the latter's ramifications.

The first of these two norms would seem to deserve preference, because it assumes a comparatively simple structure of the sporophyte, which, moreover, would differ but slightly from that of the *Bryophyta*. Apart from its independence of the gametophyte and its larger dimensions its main difference from the latter would be its division in a few subequal branches. This division is generally, although apparently on insufficient grounds, assumed to be a dichotomy, but even if it

could be proved that this is the correct interpretation, its significance should not be overrated, for the circumstance that this type of branching would be characteristic for the simply constructed sporophyte of the *Psilophytopsida*, would not necessarily mean that it is everywhere in the Vascular Plants the first step in the differentiation of the outward form. This conclusion is certainly not justified, for it is just as plausible to assume that the differentiation of the outward form may have started in the other groups of Vascular Plants by means of another type of enlargement, viz. by the development of lateral excrescences. This, at any rate, seems to be the simplest interpretation of the situation that is met with in the other groups. I know, of course, quite well that in one of these groups, viz. in the *Lycopsida*, dichotomy is the normal mode of branching, but it is not this kind of branching with which we are here concerned. This kind is to be regarded as a later phase in the development of the sporophyte; the first step is the differentiation of the lateral appendages of the originally simple axis. These lateral appendages are, of course, the so-called leaves.

The assumption that the development of the lateral mode of ramification was entirely independent of that of the dichotomous one, is on the other hand not necessary either. If the branches of the dichotomy are sufficiently unequal, the stronger one may push the weaker one aside, and placing itself in the continuation of the unbranched part, it may form with the latter a sympode. If this process was repeated a few times, the resulting sympode would be indistinguishable from a central axis provided with more or less equidistant lateral branches.

However, whether we assume that the lateral branching developed in the way described in the preceding paragraph, or that it developed independently, the first stage would have been a more or less pyramidal sporophyte with reproductive parts not only at the end of the axis but also at the end of all the lateral branches. The next step would be that the reproductive part at the end of the axis remained in abeyance, and that the latter continued its growth, producing ever more lateral branches.

A similar division of labour as took place between the axis and the lateral branches and which led to the suppression of the reproductive part at the top of the axis, may have led to the sterilisation of part of the lateral branches. The task to provide food for the growth of the sporophyte may have been restricted to some of the lateral branches, which assumed a flattened form, and which, eventually, may have reached greater dimensions, but lost the power to produce a reproductive part, whereas the lateral branches that retained this faculty, may on the other hand have shrunk. In this way the situation may have arisen that we find in the *Sphenopsida*.

The situation found in the *Lycopsida* might have arisen in a similar way; a further reduction of the branches ending in the reproductive part might have led to their complete disappearance; in this way the reproductive parts would have become sessile on the main axis. However, the circumstance that they are found in the axil of the sterile appendages, the "leaves", gives this explanation a somewhat strained

look. It would mean that the fertile and sterile branches were originally arranged in pairs, a fertile branch always just above a sterile one. To explain this curious arrangement it seems more plausible to assume either a splitting of the original lateral branches accompanied by a division of labour between the upper and the lower product of this splitting, and followed by a reduction of the upper one with as result a sessile reproductive part, or else the development of a lateral branch near the top of the original one, after which the lateral branch would take over the nutritive function, and growing in size would overtop the reproductive part; subsequent reduction of the part from which "leaf" and sporangium arise, would result in the situation that is actually observed. A similar development might also be assumed for the *Sphenopsida*, where sometimes the relation between the stalked reproductive part and the leaflike lateral appendages appears to be the same as that found in the *Lycopsida* between the sessile reproductive part and the subtending sterile appendages. The difficulties that we encounter in trying to connect the situation found in the *Lycopsida* and *Sphenopsida* with that prevailing in the *Psilophytopsida* accentuates the width of the gulf by which these groups are separated. In fact, it can not be said that the study of this aberrant group of fossil plants has shed much light on the morphology of the other Vascular Plants.

The situation found in the small group of genera formed by *Botrychium* and its allies, for which on account of its isolated position among the Vascular Plants a separate class *Botrychiopsida* should be created, may, as pointed out by Bower, be derived from that found in the *Lycopsida* by assuming a greater differentiation, and a reduction in number, of the lateral appendages of the vegetative part accompanied by a splitting of the reproductive part into a large number of "sporangia" arranged in a spike-like or thyrsoid complex.

In how far the situation found in the genera *Salvinia* and *Azolla* and that met with in *Marsilia* and its allies may be understood by comparing them with the situation observed in the preceding groups, is difficult to decide. The taxonomic position of these two groups of genera too is doubtless sufficiently isolated to justify our view that they should be regarded as distinct classes, for which the names *Salviniopsida* and *Marsiliopsida* may be used. Their inclusion in the *Filicales* and the assumption of an affinity with the in almost every respect fundamentally different *Hymenophyllaceae* and *Schizaeaceae* on account of a superficial resemblance in the structure of the "sporangia" and in the case of *Salvinia* and *Azolla* in the structure of the "sori", is so naive that one wonders how it could have been so widely accepted. In both classes the reproductive parts are split up in a number of "sporangia", a situation that we have met already in the *Botrychiopsida*, and the way in which in both classes these groups of "sporangia" are attached to a leaflike part also reminds us of the *Botrychiopsida*. Therefore it would be best to place these two classes in the vicinity of the latter. The difference in the structure of the wall of the "sporangia" is doubtless important, but its value should not be overrated. Thin walls are in sporangia not necessarily homologous structures.

The position of the reproductive parts in the *Pteropsida*, a class which I wish to restrict to the *Filicales*, seems at first sight entirely different, and is indeed difficult to explain. The structure of the "synangia" of the eusporangiate *Filicales* and the arrangement of the "sporangia" in "sori" in the leptosporangiate representatives of this group, although at first sight also rather strange, offer no great difficulties. The "synangia" find their counterpart in *Isoëtes*, which is best included in the *Lycopsida*, and the splitting of the reproductive part into a number of separate "sporangia" is met also in the *Botrychiopsida*, the *Salviniopsida* and the *Marsiliopsida*. For the position of the "synangia" and "sori" on the underside of what generally are called the "leaves" of the fern, there is in the groups that we have discussed so far, no exact counterpart. However, the circumstance that the reproductive parts occupy in all these groups a definite position with regard to a central "axis", may perhaps be taken as a hint that in this direction a solution of the problem may be found. The axis on which the so-called "leaves" are inserted, may be discarded as entirely unsuited, but in the rachis of the leaf we find an axis with regard to which the reproductive parts are indeed more or less symmetrically arranged. However, if we assume that this rachis is comparable to the central axis of the sporophyte in the *Lycopsida* and *Sphenopsida* or eventually to those parts of this axis on which the reproductive parts are found, then the so-called "leaf" of the fern must be comparable to that portion of the sporophyte of the *Lycopsida* and *Sphenopsida* on which the sporangia are borne. This conclusion, however, can not be regarded as acceptable unless we are able to answer the two following questions: 1^0 how is it to be explained that the reproductive parts are not found along the rachis itself but in some distance from the latter on the underside of the dorsiventral lamina, and 2^0 how is it that the "leaves" are so utterly different in outward form from the axial part from which they arise?

The dorsiventrality of the branch system on which the reproductive parts are inserted, and the difference in outward form between this branch system and the axis from which it arises, is after all not so very extra-ordinary, for a similar difference is found in some *Selaginella* species (e.g. *S. lepidophylla* and *S. imbricata*), where leaf-like branch systems arise from an in this case sympodial axis. The dorsiventrality of these branch systems assures in combination with their slanting position a better use of the incident light. The second question therefore does not seem to cause much difficulty.

The answer to the first question is not so easily found. It may be formulated also in this way: how can we explain the transition from a structure like the leaflike branch-system of the *Selaginella* species with the reproductive parts in the axil of the leaf-like lateral excrescences of the branches to a fern "leaf" with the reproductive parts on the underside? To explain this we should have to assume in the first place a reversion of the relative position of the reproductive part and the subtending lateral excrescence, and then the interpolation of a growth zone between the place where they are attached to the axis and the axis itself. The reversion may have been brought about by a twisting

of the primordium from which both the reproductive part and the lateral outgrowth to which it is joined, are developed. Seen in connection with the development of the dorsiventral structure, it would appear to be the most useful device to exploit the benefits of the latter to their full extent.

The explanation given in the preceding paragraph may seem to be rather far-fetched, and it might at first sight seem more plausible to assume that what we have before us in the so-called "leaf" of the ferns is no derivative situation, but an original one. However, this would not solve our difficulties, for in that case we would be compelled to derive the situation found in the *Lycopsida* and *Sphenopsida* from that in the "leaves" of the ferns, and this would require just as strained assumptions. The difficulties apparently arise from the great width of the gulf by which the *Pteropsida* are separated from the *Lycopsida* and the *Sphenopsida*.

In the *Cycadopsida*, a group that is usually included in the Phanerogams but which should be kept apart, the situation seems to be very similar to that found in the *Pteropsida*. In the vegetative part of the sporophyte a higher degree of differentiation has been achieved, the "leaves" on which the reproductive parts are inserted being reduced to scales, and the latter collected in "cones".

In the true *Phanerogamae* the situation is not so easily interpreted. The reproductive parts in which the pollen grains or microspores are produced, are borne on stalks that may resemble scale-like leaves, and are for this reason usually interpreted as leaves. The correctness of this interpretation, however, is difficult or even impossible to prove, as axillary buds, the parts by whose presence the leaves in this group are recognized, are always lacking. The morphological value of the parts by which the megasporangia or ovules are carried, is just as difficult to determine, although formerly, when our conclusions were exclusively based on the situation observed in the *Angiospermae*, it seemed absolutely certain that they were to be interpreted as leaves; in the latter group the resemblance between the parts on which the ovules are borne, the so-called carpels, and ordinary leaves is sometimes very striking. At any rate, even if it should appear that the stamens and carpels of the Phanerogams are not fully homologous with the leaves of the *Pteropsida*, it seems reasonable to assume that at least the carpels of the Angiosperms with their often fairly numerous ovules will have arisen in a similar way as the "leaves" of the *Pteropsida*, and if this applies to the carpel, there is good reason to assume that it will apply to the stamen too.

In the preceding paragraph I have expressed some doubt with regard to the existence of a full homology between the stamens and carpels of the *Phanerogamae* and the "leaves" on which in the *Pteropsida* the reproductive parts are borne. If we assume for a moment that the stamens and carpels of the *Phanerogamae* are homologous with the ordinary leaves of these plants, then the question may be formulated also in this way: are the leaves of the *Phanerogamae* homologous with the parts to which in the *Pteropsida* this name is applied?

That I put the *Phanerogamae* first in formulating the question raised in the preceding paragraph, is no accident. When they spoke of leaves, botanists thought originally of Phanerogams only, and when they afterwards extended the use of the term to ferns, they simply did not realize that it might here be misapplied. In classical morphology the leaf is defined as a lateral appendage of the stem with a bud, or eventually a shoot, in its axil. This definition is applicable only to Phanerogams, and even here it does not apply to all the parts that are usually designated as leaves, for the perianth lobes, the stamens and the carpels are never provided with buds in their axil; if the last-named parts therefore are regarded as leaves, we have to assume that the buds have aborted; this, of course, is possible. In the *Pteropsida*, however, buds are never present in the axils of the parts to which the name leaf is applied. In some distance of the latter we find the meristem patches that eventually may develop into new shoots, but the localisation of these patches is not always the same, and it is, moreover, questionable whether these entirely undifferentiated groups of cells may be homologized with the buds of the Phanerogams. In view of the totally different way in which the branches of the *Pteropsida* are formed, it seems impossible to regard them as fully homologous with the branches of the Phanerogams, and if the branches are not fully homologous, it is hardly to be expected that the leaves will be. It is rather unfortunate that in the *Cycadopsida* the mode of branching seems to be unknown. Branched stems are in this group very rare, but as the cones are usually terminal, the stems will often be sympodial, and it ought to be possible to determine the position of the bud by which the shoot is produced that will form the next member of the sympode. The results of such a study might throw new light on the affinities of this group of plants.

In the preceding considerations we have as much as possible avoided the use of the terms "stem" and "leaf". If it is true that the leaves of the *Phanerogamae*, the group in which the term "leaf" first obtained a morphological definition, are not fully homologous with the "leaves" of the ferns, and that the latter are not comparable to the "leaves" of the *Lycopsida* and *Sphenopsida* but to the whole leafy shoot of the latter, it will have to be admitted that the Phanerogams are the only group in which this term has rightly been applied, and that its use in the morphology of the other groups must necessarily lead to confusion. If it is applied at all in these groups we should realize that we do not use it in a morphological sense, but in an organographical, i.e. a functional or ecological, one.

In the *Phanerogamae* the division of the vegetative part of the sporophyte in stem and leaves has in the past proved its usefulness in the efforts to master the great diversity of detail in the plan according to which this part of the sporophyte is built. In recent times attempts have been made to replace this admittedly purely idealistic division by schemes in which the plant is regarded as consisting of a succession of identical units. The latter may consist either of a leaf with the internode at the top of which it is inserted, or else of a leaf with the sector

of the stem that extends in a downward direction to the first leaf belonging to the same vertical row. So long as these "phytonic" theories are confined to the Phanerogams, there is very little against them, but their application should not be extended to other groups of Vascular Plants, for as neither the "stem" nor the "leaves" can be regarded in the various groups as homologous parts, the combination of such a "leaf" with a portion of such a "stem" can not be homologous either. Whether these views are to be accepted in the Phanerogams instead of the classical dualistic interpretation, will depend in the first place on their adaptability to the purposes for which such an interpretation is intended, and in this respect there can, in my opinion, be no great difference between them, for if they are accepted, the next step will be that the unit is divided in a leaf, which is to be defined in the same way as before, and in a stem piece.

However, to be quite fair with regard to these "phytonic" views, we will do well to realize that the juxtaposition stem—leaves overemphasizes the importance of the stem, and that this juxtaposition has more than once led to misconceptions. In works on plant anatomy the growing point by whose activity the continuation of the stem is secured and new leaves are evolved, is usually designated as the growing point of the stem. Further, when the first rudiments of the leaves become visible at some distance from the top, the naked top portion is described as part of the stem. However, when we realize that the main part of the stem is formed by the internodes, we will have to admit that at least this part of the stem is not represented at all in the growing point. Moreover, that the leaves would arise at some distance from the top, viz. at the point where their primordia first become visible, is a gratuitous assumption. In the embryo of the *Monocotyledones* the single cotyledon is produced in a terminal position, and at the top of the embryo of a Dicotyledonous plant similarly two cotyledons are formed, and only after the latter have reached a certain size, the top becomes the starting point of a new development; the original position of the two cotyledons therefore might be described as jointly-terminal. In the *Monocotyledones*, moreover, the leaves immediately following the cotyledon are also occupying at first a terminal position, and where in the full-grown plant the leaves end in spines, as in *Aloë*, *Sanseviera*, *Agave*, the perfect radial symmetry of the top portion of these spines still bears witness to the originally terminal position of the leaf primordium, just as the dorsiventrality of the subsequently developed parts of the leaf may be accepted as proof that these parts were formed after the leaf primordium had shifted to a lateral position. The development of a new leaf primordium from one side of the old one is responsible for a shifting of the latter in the opposite direction. The stem, on the other hand, owes its origin to the expansion of the basal parts of these leaf primordia, and its subsequent development to the evolution of intercalary growth zones in this meristematic cell mass. A critical analysis of the processes that take place in the growing point therefore stresses the significance of the leaves, and favours a "phytonic" interpretation. That this has mostly been overlooked, may be due to the

circumstance that most anatomists were led astray by the study of the easily accessible growing points of *Hippuris* and *Elodea*, which to a superficial observer suggest the presence of a stem with, at a considerable distance from the top, the first signs of leaf development. This impression is entirely false. The core of such a growing point may be regarded as belonging to the stem, but there is certainly no reason whatever to see in the superficial layer at the top anything but the as yet undifferentiated stratum from which the leaves will arise. The first sign of internodes is noticed at a point that is even much farther removed from the top than the point at which the first leaf primordia become visible. In the ferns, on the other hand, there is good reason to believe that the "leaves" originate in a lateral position, and this might be another reason to deny their full homology with the leaves of the Phanerogams.

Above we have referred to a difference in position of the buds in the Phanerogams and of the meristematic patches from which in the ferns eventually new shoots may arise. The position the new shoots occupy in regard to the old ones, is doubtless not only here but everywhere in the Vascular Plants a point of morphological importance. Nevertheless it can not be regarded as equal in importance to the study of the position the reproductive parts occupy in relation to the vegetative part, for the division of the sporophyte in a vegetative and a reproductive part is a feature that is found in all *Embryophyta*, and for this reason these parts must occupy in the general plan of this group a definite position with regard to each other, of which eventual deviations are to be explained by the aid of auxiliary hypotheses, but the branching of the vegetative part is evidently confined to one of the subdivisions of this group, viz. to the Vascular Plants, and as it evidently arose in most groups only after the vegetative part had reached a considerable degree of differentiation, it seems reasonable to assume that it will have developed independently in the various groups.

Before entering into this subject I should like to make a few remarks on the use of the term "branching". In discussing the primary differentiation of the vegetative part I have used it, instead of a better one, for those processes which lead to an enlargement of this part by the production of the kind of appendages that, on account of a functional similarity, are usually designated as "leaves", but which, as we have seen, may possess a quite different morphological value. However, as a rule the use of the term is confined to such processes of enlargement in which the new-formed parts are a more or less faithful replica of the original one, and in this sense we will use it in the following considerations.

The kind of branching defined in the preceding paragraph may take place in two ways. It may be due to a splitting of the growing point into two more or less equal or, rarely, distinctly unequal parts, but the branches may also arise from groups of meristem cells that are set aside for this purpose at some distance from the apex of the growing point, and that for some time remain dormant. In the first case we speak of dichotomy, in the second case of lateral branching.

Dichotomy is the typical mode of branching in the *Lycopsida*. Here the equivalence of the two branches is reflected in their symmetrical position with regard to the uppermost "leaf" of the axis from which they arise; this "leaf" appears to be inserted just below the fork. In this typical form dichotomy appears to be confined to the *Lycopsida*, and here it is the only mode of branching. A forking of the stem occurs, however, also in the leafless *Psilophytopsida*, where, however, it is not certain that it is a true dichotomy, and further, by way of exception, in some Phanerogams. In this class it has been observed as a regular feature in some species of the palm genus *Hyphaene*, and as a monstrosity in some other *Monocotyledones* (*Fritillaria meleagris*, *Tulipa*). In the *Hyphaene* species investigated by SCHOUTE (Rec. d. trav. bot. Néerl. 6: 1. 1909) the uppermost leaf below the fork was found to occupy the same position as the corresponding "leaf" in the *Lycopsida*, but in another *Hyphaene* species which I myself could study (Rec. d. trav. bot. Néerl. 25A: 75. 1928), this position was occupied by the highest leaf but one, so that the position of the uppermost leaf below the fork can not be regarded as constant. This seems to accentuate the more or less accidental character of this type of branching in this genus, where I regarded it (l.c. p. 79) as a "very regular, hereditary fasciation", comparable to the forking occasionally observed in fasciated stems of *Fritillaria meleagris* and *Tulipa*, and therefore of minor importance. It should furthermore not be overlooked that it is in these species of *Hyphaene* by no means, as in the *Lycopsida*, the only mode of branching, for the inflorescences develop from axillary buds.

In the *Sphenopsida* the branching is always lateral, but the position of the branches with regard to the "leaves" differs from that observed in the *Pteropsida* and in the *Phanerogamae*, for they are found to alternate with them. This entirely different arrangement doubtless points to an independent development.

The differences in the origin of the lateral branches in the *Pteropsida* and the *Phanerogamae* have already been mentioned.

The ideas set forth in the preceding pages might have been expressed also in a phylogenetic form. We might have started e.g. from the *Bryophyta* with their simple sporophyte provided with a single terminal reproductive part. From such a type the *Psilophytopsida* might have sprung, where we find forked stems with a reproductive part at the end of the ramifications. The next step might have led to the *Sphenopsida*, where the terminal reproductive part has been suppressed, but where the function of the latter has been taken over by the reproductive parts at the end of the ramifications of the vegetative part, of which a large part has been modified into "leaves". Further reduction of the lateral excrescences ending in reproductive parts would have led to the situation found in the *Lycopsida*. A concrescence of all the lateral parts would have produced the "leaf" of the *Pteropsida*, and from the situation found in the latter we might have passed on to the *Cycadopsida* and the *Phanerogamae*.

This phylogenetic system has, like all the other ones that have been

proposed, several weak points, of which I will mention but one, viz. the assumption that the starting point should be found in the *Bryophyta*. We might just as well have chosen the fern leaf as our starting-point, i.e. a vegetative part provided with a considerable number of reproductive parts that are more or less evenly scattered over its whole surface. This would have had the advantage that we would have started from a sporophyte that was built according to the same plan as the gametophyte, where we find as a rule a large number of reproductive parts (antheridia and archegonia) scattered over the vegetative part. Starting from this point we would have recognized in the situation met with in the *Bryophyta* one of extreme reduction. In the absence of decisive fossil evidence—and there is very little hope that the latter will ever be forthcoming—, such a view could be defended just as well.

In idealistic morphology it is of no great importance from which point we start, as it confines itself to establishing homologies and distinguishing between various modifications of the homologous parts. In the Vascular Plants the latter prove to be of greater importance than the homologies themselves, which means that a morphology of the various classes is more promising than one of the whole group. However, before engaging in a morphology of one of these groups, one should be quite clear with regard to the meaning of the terms that are to be used. One of the aims of this paper is to show that such terms as "leaf" and "stem" should be avoided, as the parts to which they have so far been applied in the different groups, are of entirely different morphological value.